UNIVERSITI PUTRA MALAYSIA

COMPUTATIONAL APPROACHES TO ELUCIDATE THE ACTION MECHANISM OF ZERUMBONE TOWARDS BETA CATENIN

AYESHA FATIMA

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By

AYESHA FATIMA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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"It always seems impossible until it's done"

-----Nelson Mendela (1918-2013)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

COMPUTATIONAL APPROACHES TO ELUCIDATE THE ACTION MECHANISM OF ZERUMBONE TOWARDS BETA CATENIN

By

AYESHA FATIMA

August 2017

Chairman : Associate Professor Ahmad Bustamam Abdul, PhD
Institute : Bioscience

Cancer is the fourth leading cause of all deaths in Malaysia with 13.56% deaths due to cancer in 2014. Wnt-β-catenin signaling pathway plays a significant role in cell proliferation and migration. Due to this, it has been implicated in several human cancers. β-catenin is the key protein of the Wnt-β-catenin signaling pathway that is involved in maintaining the cell-cell adhesion by binding to the e-cadherin and α-catenin and cell proliferation by complexing with transcription factor, Lymphoid Enhancing Factor 1 (LEF1)/T-cell Factor 4 (TCF4), in the nucleus. Zerumbone is a sesquiterpine with known anticancer properties. The action of zerumbone towards β-catenin was investigated using several computational techniques.

Docking studies was done with CDOCKER. Zerumbone was docked to β-catenin with and without TCF4 to represent structures of β-catenin in the nucleus and in the cytoplasm, respectively. Binding energy of -60.58 kcal/mole was obtained when zerumbone was docked to the β-catenin without TCF4. Trp338, Arg342, Lys345, Arg376, Asn380 and Trp383 formed the binding site residues. When zerumbone was docked to the β-catenin-TCF4 complex, it interacted with residues of both β-catenin and TCF4. The binding energy of the β-catenin-TCF4-zerumbone complex was -80.8 kcal/mol and the binding pocket residues were Lys345, Trp383, Arg386, Asn387, Asn415 of β-catenin and residues Glu24, Gly25, Gln27, Glu28 of TCF4. The results indicated that zerumbone was bound more strongly to the β-catenin-TCF4 complex than β-catenin without TCF4.

The stability of the β-catenin-zerumbone and β-catenin-TCF4-zerumbone complexes was futher investigated by molecular dynamics simulation technique implemented in AMBER12 for 50ns. The β-catenin-zerumbone complex with RMSD values between 3-6Å indicated an effort in forming a stable complex. The β-catenin-TCF4-zerumbone complex, on the other hand, with RMSD values between 4-5Å demonstrated the formation of a stable complex. The MMPBSA/GBSA methods used for calculating the average free binding energy of the last 5ns simulation the complexes showed that the PBTOTAL and GBTOTAL values of the β-catenin-zerumbone complex were -7.94 kcal/mol and -7.54 kcal/mol, respectively. The same values for the β-catenin-TCF4-zerumbone complex were -15.23 kcal/mol and -14.88 kcal/mol, respectively.

The hybrid QM/MM molecular dynamics provided deeper insight into the binding site interactions of the β-catenin-TCF4-zerumbone complex. The binding site of zerumbone with the key residues Lys345, Trp383, Arg386 of β-catenin and Gln27 and Glu28 of TCF4 was treated by QM PM6 semi-empirical theory, while the remaining protein was treated with MM theory using AMBER12. The 5ns simulation data showed that the RMSD of the QM treated region was between 2-2.5 Å. The free binding energy of the QM treated region was -15.68 kcal/mol illustrated that zerumbone was tightly bound to the complex. The structural data indicated that the ring structure of zerumbone formed π-π interactions with the aromatic rings of Trp383 of β-catenin while Arg386 also from β-catenin formed hydrogen bond with the carbonyl oxygen of zerumbone lending stability to the binding of zerumbone to the complex. Steered molecular dynamics method in GROMACS 5.0.4 software was used to estimate the strength of interaction of the β-catenin-TCF4 complex in the presence of zerumbone. The results indicated that the β-catenin-TCF4 complex interaction was 1963.1 kJ/mol/nm which was considerably less when compared to 2221.9 kJ/mol/nm when zerumbone. *In-vitro* experiments conducted on HepG2 cell lines demonstrated that the nuclear: cytoplasmic ratio of β-catenin decreased significantly at 8μg/mL indicating decreased translocation of β-catenin into the nucleus.

In conclusion from our investigation, zerumbone targets β-catenin in the cytoplasm as well as in the nucleus when it is bound to the transcription cofactor, TCF4.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENDEKATAN BERKOMPUTER UNTUK MENJELASKAN MEKANISME TINDAKAN ZERUMBON TERHADAP β-KATENIN

Oleh

AYESHA FATIMA

Ogos 2017

Pengerusi : Profesor Madya Ahmad Bustamam Abdul, PhD
Institut : Biosains

Kanser merupakan penyebab utama kematian yang keempat di Malaysia, iaitu 13.56%, pada tahun 2014. Laluan isyarat Wnt-ß-catenin memainkan peranan yang penting dalam percambahan sel dan migrasi. Ia terbabit dalam beberapa kanser manusia. ß-catenin ialah protein utama dalam laluan isyarat Wnt-ß-catenin yang boleh mengekalkan kesegahan sel dengan cara mengikat pada e-cadherin dan α-catenin, serta percambahan sel menerusi pembentukan kompleks dengan faktor transkripsi Lymphoid Enhancing Factor 1 (LEF1)/T-cell Factor (TCF4) dalam nukleus. Zerumbone ialah seskuiterpen yang memiliki sifat antikanser. Kesan zerumbone terhadap ß-catenin telah diselidik dengan beberapa teknik komputasi.

CDOCKER digunakan untuk kajian mengedok. Zerumbone telah didokkan ke ß-catenin, dengan/tanpa TCF4 untuk mewakili struktur ß-catenin dalam nukleus dan sitoplasma. Energi pengikatan sebanyak -60.58 kcal/mol dilaporkan apabila zerumbone didokkan ke ß-catenin, tanpa TCF4. Di tapak pengikatannya, terdapat residu-residu asid amino seperti Trp338, Arg342, Lys345, Arg376, Asn380 dan Trp383. Interkasi juga berlaku antara zerumbone dengan ß-catenin-TCF4 kompleks. Energi pengikatannya adalah sebanyak -80.8 kcal/mol. Lys345, Trp383, Arg386, Asn387, Asn415 dari ß-catenin dan Glu24, Gly25, Gln27, Glu28, dari TCF4 adalah antara residu-residu yang terlibat dalam poket pengikatan tersebut. Ini menunjukan bahawa zerumbone mampu mengikat pada kompleks ß-catenin-TCF4 dengan lebih kuasa, berbanding dengan ß-catenin tanpa TCF4.

Kestabilan ß-catenin-zerumbone dan kompleks ß-catenin-TCF4-zerumbone juga dikaji menggunakan teknik simulasi dinamik molekul sepanjang 50ns dalam AMBER12. Nilai-nilai RMSD antara 3-6 Å bagi kompleks ß-catenin-zerumbone menunjukkan

kemampuannya untuk membentuk kompleks yang stabil. Di sebaliknya, kompleks ß-catenin-TCF4-zerumbone memiliki nilai-nilai RMSD antara 4-5 Å dan formasi kompleks tersebut juga adalah stabil. Pengiraan purata energi bebas pengikatan telah dilakukan untuk 5 ns terakhir simulasi dan nilai-nilai PBTOTAL (cara MMPBSA) dan GBTOTAL (cara GBSA) bagi kompleks ß-catenin-zerumbone adalah masing-masing, -7.94 kcal/mol dan -7.54 kcal/mol. Bagi kompleks ß-catenin-TCF4-zerumbone, nilai-nilai PBTOTAL dan GBTOTAL adalah masing-masing, -15.23 kcal/mol dan -14.88 kcal/mol.

Disebabkan oleh kestabilan kompleks ß-catenin-TCF4-zerumbone yang tinggi, perhitungan hybrid QM/MM dijalankan untuk memahami interaksi di tapak pengikatan dengan lebih mendalam. Residu-residu utama di tapak pengikatan seperti Lys345, Trp383, Arg386 dari ß-catenin dan Gln27, Glu28 dari TCF4 dikaji dengan kaedah QM semi empirikal PM6, manakala residu-residu lain dikaji dengan kaedah MM menerusi AMBER12. Data simulasi 5 ns menunjukkan nilai-nilai RMSD antara 2-2.5 Å di kawasan yang dikaji dengan kaedah QM. Energi bebas pengikatannya adalah sebanyak -15.68 kcal/mol dan zerumbone terikat ketat dalam kompleks tersebut. Dalam analisis data struktural, gelang zerumbone membentuk interaksi π-π dengan gelang aromatik Trp383 dari ß-catenin manakala Arg386 membentuk bon hidrogen dengan karbonyl oksigen zerumbone, untuk menstabilkan pengikatan zerumbone pada kompleks. Bagi memahami kesan zerumbone yang terikat pada kompleks ß-catenin-TCF4, kaedah dinamik molekul 'steered' dalam GROMACS 5.0.4 digunakan untuk menganggar kuasa interaksi kompleks ß-catenin-TCF4 dengan zerumbone. Jumlah daya untuk menarik rantai TCF4 dari ß-catenin adalah 1963.1 kJ/mol/nm, lebih kurang daripada kompleks tanpa zerumbone yang memiliki jumlah daya sebanyak 2221.9 kJ/mol/nm.

Eksperimen *in vitro* bagi titisan sel HepG2 menunjukkan bahawa nisbah nuklear:sitoplasmik ß-catenin menjadi kurang dengan nyata sekali pada 8 µg/mL. Ini juga menjelaskan kekurangan pergerakan ß-catenin terhadap nukleus.

Secara keseluruhannya, zerumbone sesuai untuk menyasar ß-catenin ke dalam sitoplasm dan nukleus apabila terikat pada kofaktor transkripsi, TCF4.

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I certify that a Thesis Examination Committee has met on 9 August 2017 to conduct the final examination of Ayesha Fatima on her thesis entitled "Computational Approaches to Elucidate the Action Mechanism of Zerumbone Towards Beta Catenin" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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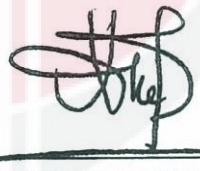

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Name of Member of Supervisory Committee: Professor Dr. Rasedee Abdullah

Signature: _____
Name of Member of Supervisory Committee: Dr. Roghayeh Abedi Karjiban

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Vannajan Sanghiran Lee

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Wnt	Wingless integrase
Dsh	Disheveled
APC	Adenomatous polyposis coli
TCF	T-cell factor
LEF	Lymphoid enhancer binding factor
GSK3b	Glycogen synthase kinase 3 b
LRP	5/6 Lipoprotein receptor related protein 5/6
CK1	Casein kinase 1
SAMP	Serine-Alanine-Methionine-Proline
PP2A	Protein phosphatase 2 A
PSD-95	Post-synaptic density protein 95
Dlg1	Drosophila disc large tumor suppressor
ZO-1	Zona occludens 1
WTX	Wilms tumour suppressor
AMER1	APC membrane recruitment 1
PIP2	Phosphatidylinositol 4,5-biphosphate
CDK2	Cyclin D kinase 2
FZD	Frizzled
NRH1	Neutrophin receptor homolog 1
PTK7	Protein tyrosine kinase like 7
ROR2	Receptor tyrosine kinase-like orphan receptor 2
DAAM1	Dishevelled-associated activator of morphogenesis 1
CamKII	Calcium/calmodulin-dependent kinase II
TAK1	TGFβ activated kinase
TGFβ	Transforming growth factor β
NLK	Nemo-like kinase

CDC42	Cyclin dependent kinase 42
PCP	Planar cell polarity
NLS	Nuclear localisation signal
NES	Nuclear export signal
NPC	Nucleoprotein complex
HEAT	Huntington, elongation factor 3, protein phosphatase 2A, yeast kinase TOR1
CRM1	Chromosomal Maintenance 1
ARM	Armadillo
WRE	Wnt responsive element
BCL9	B-cell lymphoma 9 protein
Pygo	Pygopus
CRBP	cAMP-response element-binding protein
HATs	histone acetyltransferases
TRRAP	Transformation/TranscriptionDomain Associated Protein
HMT	Histone methyltransferases
DDK1	Dickkopf-1
HER	Human Estrogen Receptor
EGFR	Epidermal Growth Factor Receptor
PI3K	Phosphoinoistide 3-kinase
MMP7	Matrix metallopeptidase-7
SFRP	Soluble Frizzled Receptor Protein
WIF	Wnt inhibitory factor
TANKS	Tankyrases
IWR-1	Inhibitor of Wnt response 1
iCRT14	Inhibitor of β-Catenin Response Transcription14
RANKL	Receptor activator of Nuclear factor kappa-B ligand
NF-κB	Nuclear factor-kappa B
IkKa	Inhibitor of kappa β alpha kinase

TRAIL	Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand
DR5	Death receptor 5
HSP	Heat Shock Protein
IL-1b	Interleukin 1b
TNF-α	Tumour Necrosis Factor alpha
MAP	Microtubule Associated Protein
MMPBSA	Molecular Mechanics Poisson Boltzmann Surface Area
MMGBSA	Molecular Mechanics Generalised Born Surface Area
CADD	Computer aided drug design
CAMD	Computer aided molecular design
SBDD	Structure based Drug Discovery and Designing
AMBER	Assisted Model Building and Energy Refinement
QM	Quantum mechanical or mechanics
DFT	Density Functional Theory
CNDO	Complete neglect of differential overlap
INDO	Intermediate neglect of differential overlap
AM1	Austin Model 1
PM3	Parametrised Model 3
PM6	Parametrised Model 6
SMD	Steered Molecular Dynamics
AFM	Atomic Force Microscopy
CHARMM	Chemistry at Harvard Molecular Mechanics
PMF	Potentials of Mean Force
VDW	van Der Waals
TNFR1	Tumour Necrosis Factor Receptor 1
JN	c-Jun N-terminal Kinase
NIK	Nuclear Factor kappa B Inducing Kinase
RMSD	Root Mean Square Deviations

MD	Molecular Dynamics
Zer	Zerumbone
ALT	Alanine Transaminase
AST	Aspartate Transaminase
AP	Alkaline phosphatase
AFP	Alpha-fetoprotein
PCNA	Proliferating cell nuclear antigen
ID2	Inhibitor of DNA-binding protein 2
BIRC5	Baculoviral Inhibitor of Apoptosis Repeat-Containing Protein 5
HELLS	Helicase Lymphoid Specific
MCTS1	Malignant T-cell Amplified Sequence 1
TERT	Telomerase Reverse Transcriptase
SMC2	Structural Maintenance of Chromosome Protein 2
AXIN2	Axis Inhibition P*rotein* 2
AURKA	Aurora Kinase A

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer is a non-communicable disease that causes death worldwide. According to the recent report published by the Malysian National Cancer Registry, 103,507 new cases of cancer were diagnosed between 2007-2011. The top five cancers affecting the Malaysian population are breast cancer (17.7%), colorectal cancer (13.2%), trachea, bronchus, lung cancer (10.2%), lymphoma (5.2%) and nasopharyngeal cancer (4.9 %). Liver cancer is less common with 4% occurrence rate [1] .

Cancer can result from variety of factors including genetic mutations and environmental factors. Because of the tight regulatory systems in the human body, faulty signaling pathways that lead to persistent cell division and growth are considered as one of the causes of cancer. Almost all signaling pathways influence normal cell growth. Due to the eminent and probable cellular signaling involved in crosstalk, it is crucial to understand signaling pathways implicated in cancer growth and to possibly intervene these pathways for better prognosis of cancer.

The Wnt-signaling pathway is a central regulating signaling for cell differentiation and growth cascade. This cellular pathway is the key in controlling functions of normal and malignant epithelial cells and currently has become an important new targets for anti-cancer drug development [2–4]. Its importance in cancer development and possibly metastasis has attracted researchers globally to dedicated efforts on investigating this pathway [5–8]. Hence, large amount of information with regards to the cause of cancer due to this pathway has since been accumulated. The central protein of this pathway, β-catenin together with its regulation is one of the most important targets of current development of newer generations of anti-cancer drugs.

The fight against cancer ranges from designing selective targeted inhibitors of known aberrant proteins to preventing faulty cell cycle signaling, which continuously produces unwarranted cancerous cells. Several drugs from natural and synthetic sources are currently being discovered and investigated in these aspects.

Zerumbone, molecular formula $C_{15}H_{24}O$, a natural compound extracted and isolated from *Zingiber zerumbet* Roscoe, has been the focus of anti-cancer research for nearly two decades. Its effectiveness and probable mechanism of actions by which it prevents cancer progression has been the topic of extensive experimentation over the years being investigated [9]. Zerumbone possesses anti-inflammatory, antibacterial, and anti-cancer

properties [9–11]. It is an eleven membered monocyclic sesquiterpene with an α,β-unstaurated carbonyl group. Its molecular weight is 218 Da. The chemical structure is given in Figure 1.1.

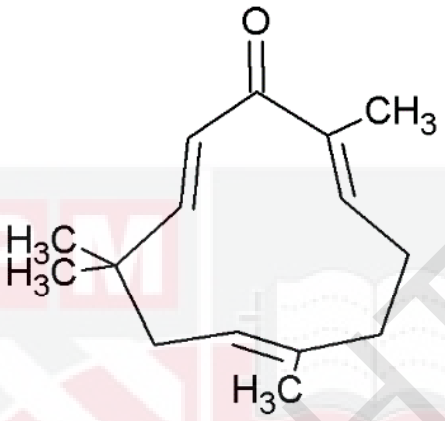

Figure 1.1 : Chemical structure of Zerumbone

The α,β-unstaurated carbonyl group of zerumbone allows it to form Michael adducts with target molecules [12,13]. Absence of this group renders it ineffective [14]. Also its ring structure makes it hydrophobic in nature. Its structure was elucidated by Hall et al. (1981). The compound has three double bond, viz between C2-C3, C6-C7 and C10-C11 which render the molecule chiral as well as able to bend despite the inherent rigidity of ring structure [15] Literature survey has shown, that it target proteins directly that are part of the NF-κB complex and induces apoptosis by increasing the expresssion of other proteins in a pleiotropic manner [16–18]. Due to multitude of signaling pathways involved in cancer, it remains to be seen whether zerumbone is selective towards proteins of the Wnt-β-catenin pathway also.One fundamental aspect of modern drug discovery today is the extensive use of computers for predicting the physicochemical, stereochemistry as well as probable biological entity as useful drugs for future therapeutics. The method is popular since the cost to market a new therapeutic drug could be worth millions and a waiting period of at least ten years for profiling its clinical effectiveness in human trials is forcing most global conglomerate pharmaceutical companies to acquire better efficient methods to substantially decrease the drug hit-to-lead-to-synthesis-to formulation time frame. This could probably lead to a more focused effort in determining an exact drug for further extensive *in-vivo* studies and human clinical trials. In this respect, current usage in computational experimentation is found to be cost effective to discover newer and potent drugs for commercial production. This has created all related procurement in drug discovery to be made possible using computers, and therefore investments in pharmaceuticals has currently shifted on developing faster, newer and more accurately predicting algorithm implemented using high throughput computers [19–21].

1.2 Significance of the study

As mentioned above, unregulated signaling pathways can be a cause cancer. Therapeutic intervention is required to inhibit this dysregulation. Zerumbone inhibits one of these pathways, the NF-κB pathway [18]. However, there are several other dysregulated pathways such as the Wnt-β-catenin pathway that can act as parallel mechanisms for the continued growth of cancerous cells. Hence, the current investigation is important due to the following reasons.

Aberrant Wnt-β-catenin pathway is known to be one of the causative pathways in cancer and β-catenin is one of the major proteins responsible in cancerous cell proliferation especially in colonic, hepatic, pancreatic cancers and leukaemia [6,8,22–26]. Presently there is no commercially available anti-cancer targeting the Wnt-β-catenin pathway.Zerumbone is known to inhibit colonic, hepatic, ovarian, breast cancer and leukaemia by inducing apoptosis in cancerous cells in time and concentration dependent manner [9]. There is no current investigation reported elsewhere that zerumbone intervenes in the Wnt-β-catenin pathway. No study has been done on studying the mechanism of zerumbone on β-catenin-TCF4 transcription factor complex that is most active in cancer cells. It is worth investigating if zerumbone could inhibit more than one signalling pathways involved in cancer. In that case, zerumbone can be a significant breakthough anticancer therapy suitable for further investigation.

1.3 Aims and objectives of this study

The aim of the current research is to use suitable computational softwares and simulation studies, and to relate these findings *in vitro*, in order to explore the binding mode of zerumbone in the Wnt-β-catenin pathway.

Hence, the objectives of the current study are as follows:

1. To determine whether zerumbone targets the β-catenin protein and chatacterise the binding mode of zerumbone on β-catenin using molecular docking methods.
2. To identify the structure, flexibility, and dynamics of cytoplasmic zerumbone-beta catenin and zerumbone-β-catenin-T-cell factor 4 nuclear complex using molecular dynamics simulations.
3. To determine the binding mode of zerumbone with β-catenin protein using high level of calculation using hybrid quantum mechanical / molecular mechanical dynamic simulations.
4. To establish the strength of the β-catenin-T-cell factor 4 nuclear complex in presence of zerumbone using the steered molecular dynamics.
5. To experimentally confirm the computationally predicted inhibitory effect of zerumbone on β-catenin on the liver cancer (HepG2) cell line.

To achieve the above objectives, the following research plan has been formalized as presented in Figure 1.2.

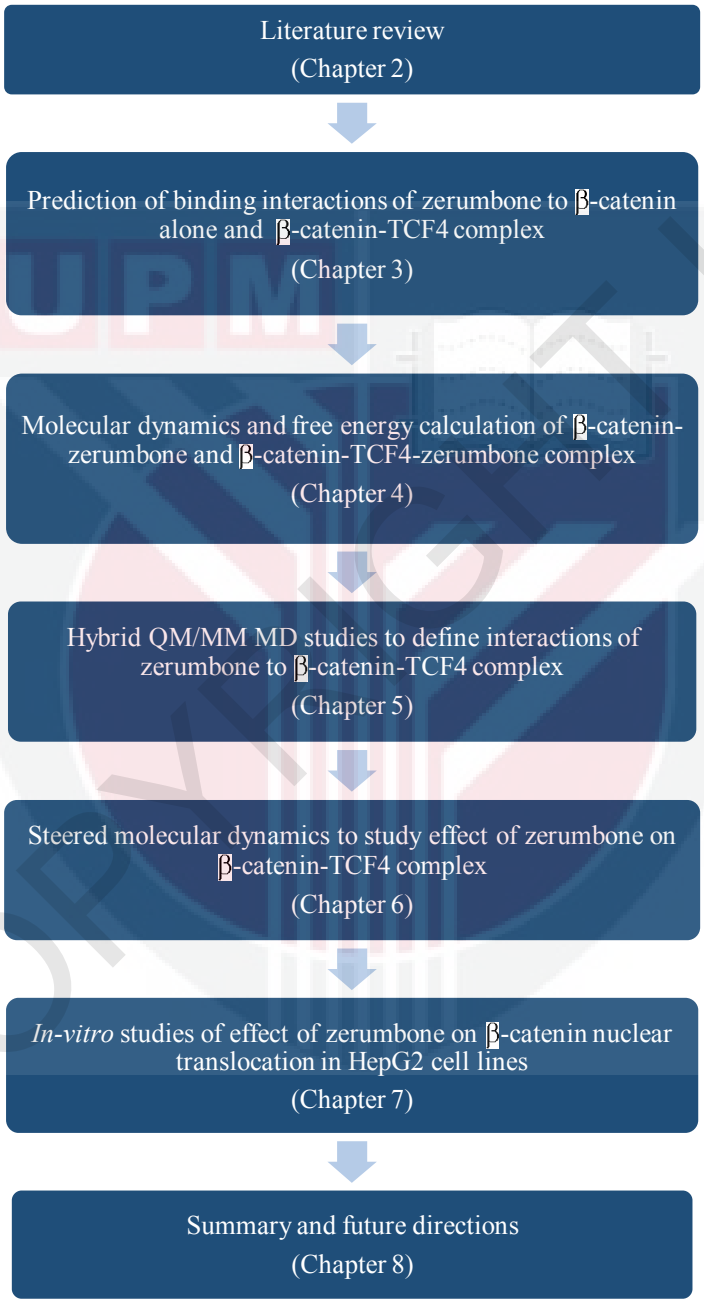

Figure 1.2 : Research plan for the study

REFERENCES

[1] Ministry of Health Malaysia, National Cancer Registry Report, 2011.

[2] R.M. Hallett, M.K. Kondratyev, A.O. Giacomelli, A.M.L. Nixon, A. Girgis-Gabardo, D. Ilieva, J. a Hassell, Small molecule antagonists of the Wnt/β-catenin signaling pathway target breast tumor-initiating cells in a Her2/Neu mouse model of breast cancer., PLoS One. 7 (2012) e33976.

[3] A. Voronkov, S. Krauss, Wnt/beta-catenin signaling and small molecule inhibitors., Curr. Pharm. Des. 19 (2013) 634–64.

[4] W. Chen, M. Chen, L.S. Barak, Development of small molecules targeting the Wnt pathway for the treatment of colon cancer: a high-throughput screening approach., Am. J. Physiol. Gastrointest. Liver Physiol. 299 (2010) G293-300.

[5] W. Lu, C. Lin, M.J. Roberts, W.R. Waud, G.A. Piazza, Y. Li, Niclosamide suppresses cancer cell growth by inducing Wnt co-receptor LRP6 degradation and inhibiting the Wnt/β-catenin pathway., PLoS One. 6 (2011) e29290.

[6] S. Fatima, N.P. Lee, J.M. Luk, Dickkopfs and Wnt/β-catenin signalling in liver cancer., World J. Clin. Oncol. 2 (2011) 311–25.

[7] Y. Duchartre, Y.-M. Kim, M. Kahn, The Wnt signaling pathway in cancer, Crit. Rev. Oncol. / Hematol. 99 (2016) 141–149.

[8] P. Polakis, The many ways of Wnt in cancer, Curr. Opin. Genet. Dev. 17 (2007) 45–51.

[9] R. Prasannan, K.A. Kalesh, M.K. Shanmugam, A. Nachiyappan, L. Ramachandran, A.H. Nguyen, A.P. Kumar, M. Lakshmanan, K.S. Ahn, G. Sethi, Key cell signaling pathways modulated by zerumbone: role in the prevention and treatment of cancer., Biochem. Pharmacol. 84 (2012) 1268–1276.

[10] M. Kim, S. Miyamoto, Y. Yasui, T. Oyama, A. Murakami, T. Tanaka, Zerumbone, a tropical ginger sesquiterpene, inhibits colon and lung carcinogenesis in mice., Int. J. Cancer. 124 (2009) 264–271.

[11] B. Sung, A. Murakami, B. Oyajobi, B. Aggarwal, Zerumbone abolishes RANKL-induced NF-κB activation, inhibits osteoclastogenesis, and suppresses human breast cancer–induced bone loss in athymic nude mice, Cancer Res. 69 (2009) 1477–84.

[12] K.A. Kalesh, J.A. Clulow, E.W. Tate, Target profiling of zerumbone using a novel cell-permeable clickable probe and quantitative chemical proteomics, Chem. Commun. 51 (2015) 5497–5500.

[13] A. Murakami, D. Takahashi, T. Kinoshita, K. Koshimizu, H.W. Kim, A. Yoshihiro, Y. Nakamura, S. Jiwajinda, J. Terao, H. Ohigashi, Zerumbone , a Southeast Asian ginger sesquiterpene , markedly suppresses free radical generation , proinflammatory protein production , and cancer cell proliferation

accompanied by apoptosis : the α , β-unsaturated carbonyl group is a prerequisite, Carcinogenesis. 23 (2002) 795–802.

[14] T. Kitayama, K. Yamamoto, R. Utsumi, M. Takatani, R.K. Hill, Y. Kawai, S. Sawada, T. Okamoto, Chemistry of zerumbone. 2. Regulation of ring bond cleavage and unique antibacterial activities of zerumbone derivatives., Biosci. Biotechnol. Biochem. 65 (2001) 2193–2199.

[15] S.R. Hall, S. Nimgirawath, C.L. Raston, A. Sittatrakul, S. Thadaniti, N. Thirasasana, A.H. White, Crystal structure of zerumbone [(E,E,E)-2,6,9,9-Tetramethylcycloundeca-2,6,10-trien-1-one], Aust. J. Chem. 34 (1981) 2243–2247.

[16] H. Ichikawa, Y. Nakamura, Y. Kashiwada, B.B. Aggarwal, Anticancer drugs designed by mother nature: ancient drugs but modern targets., Curr. Pharm. Des. 13 (2007) 3400–3416.

[17] S. Yodkeeree, B. Sung, P. Limtrakul, B.B. Aggarwal, Zerumbone Enhances TRAIL-Induced Apoptosis through the Induction of Death Receptors in Human Colon Cancer Cells: Evidence for an Essential Role of Reactive Oxygen Species, Cancer Res. 69 (2010) 6581–6589.

[18] Y. Takada, A. Murakami, B.B. Aggarwal, Zerumbone abolishes NF-kappaB and IkappaBalpha kinase activation leading to suppression of antiapoptotic and metastatic gene expression, upregulation of apoptosis, and downregulation of invasion., Oncogene. 24 (2005) 6957–6969.

[19] A.R. Ortiz, P. Gomez-Puertas, A. Leo-Macias, P. Lopez-Romero, E. Lopez-Viñas, A. Morreale, M. Murcia, K. Wang, Computational approaches to model ligand selectivity in drug design., Curr. Top. Med. Chem. 6 (2006) 41–55.

[20] I.M. Kapetanovic, Computer-aided drug discovery and development (CADDD): in silico-chemico-biological approach, Chem Biol Interact. 171 (2009) 165–176.

[21] Y.Y. Li, J. An, S.J.M. Jones, A computational approach to finding novel targets for existing drugs, PLoS Comput. Biol. 7 (2011) e1002139.

[22] P. Polakis, Wnt signaling and cancer, Genes Dev. 14 (2000) 1837–1851.

[23] P. Polakis, Wnt signaling in cancer, Cold Spring Harb. Perspect. Biol. 4 (2012) 9.

[24] J.N. Anastas, R.T. Moon, WNT signalling pathways as therapeutic targets in cancer, Nat Rev Cancer. 13 (2013) 11–26.

[25] R.K. Gandhirajan, P.A. Stalb, K. Minke, I. Gehrke, G. Plickert, A. Schlösser, E.K. Schmitt, M. Hallek, K. Kreuzer, Small Molecule Inhibitors of Wnt / β - Catenin / Lef-1 Signaling Induces Apoptosis in Chronic Lymphocytic Leukemia Cells, Neoplasia. 12 (2010) 326–335.

[26] R. Lamb, M.P. Ablett, K. Spence, G. Landberg, A.H. Sims, R.B. Clarke, Wnt Pathway Activity in Breast Cancer Sub-Types and Stem-Like Cells, PLoS One.

8 (2013) 1–11.

[27] L. Howe, A. Brown, Wnt signaling and breast cancer, Cancer Biol. Ther. (2004) 36–41.

[28] B. MacDonald, K. Tamai, X. He, Wnt/β-catenin signaling: components, mechanisms, and diseases, Dev. Cell. 17 (2009) 9–26.

[29] C.Y. Janda, D. Waghray, A.M. Levin, C. Thomas, K.C. Garcia, Structural basis of Wnt recognition by Frizzled., Science. 337 (2012) 59–64.

[30] R. Nusse, H.E. Varmus, Wnt genes., Cell. 69 (1992) 1073–1087.

[31] K. Willert, R. Nusse, Wnt proteins., Cold Spring Harb. Perspect. Biol. 4 (2012) 1–13.

[32] A.J. Mikels, R. Nusse, Wnts as ligands: processing, secretion and reception, Oncogene. 25 (2006) 7461–7468.

[33] T. Buechling, V. Chaudhary, K. Spirohn, M. Weiss, M. Boutros, p24 proteins are required for secretion of Wnt ligands, EMBO Rep. 12 (2011) 1265–1272.

[34] K.M. Cadigan, R. Nusse, Wnt signaling: a common theme in animal development., Genes Dev. 11 (1997) 3286–3305.

[35] M.D. Gordon, R. Nusse, Wnt signaling: Multiple pathways, multiple receptors, and multiple transcription factors, J. Biol. Chem. 281 (2006) 22429–22433.

[36] R. Nusse, Wnt signaling., Cold Spring Harb. Perspect. Biol. 4 (2012) a011163.

[37] H. Clevers, R. Nusse, Wnt/β-catenin signaling and disease., Cell. 149 (2012) 1192–1205.

[38] N.-C. Ha, T. Tonozuka, J.L. Stamos, H.-J. Choi, W.I. Weis, Mechanism of phosphorylation-dependent binding of APC to beta-catenin and its role in beta-catenin degradation., Mol. Cell. 15 (2004) 511–21.

[39] J.L. Stamos, W.I. Weis, The β-catenin destruction complex, Cold Spring Harb. Perspect. Biol. 5 (2013) 1–16.

[40] L. Arce, K.T. Pate, M.L. Waterman, Groucho binds two conserved regions of LEF-1 for HDAC-dependent repression., BMC Cancer. 9 (2009) 159.

[41] R.A. Cavallo, R.T. Cox, M.M. Moline, J. Roose, G.A. Polevoy, H. Clevers, M. Peifer, A. Bejsovec, Drosophila TCF and Groucho interact to repress Wingless signalling activity., Nature. 395 (1998) 604–608.

[42] K. Tanneberger, A.S. Pfister, K. Brauburger, J. Schneikert, M. V Hadjihannas, V. Kriz, G. Schulte, V. Bryja, J. Behrens, Amer1/WTX couples Wnt-induced formation of PtdIns(4,5)P(2) to LRP6 phosphorylation, EMBO J. 30 (2011) 1433–1443.

[43] A. Valbuena, A.M. Vera, J. Oroz, M. Menéndez, M. Carrión-Vázquez, Mechanical properties of β-catenin revealed by single-molecule experiments.,

Biophys. J. 103 (2012) 1744–52.

[44] W. Xu, D. Kimelman, Mechanistic insights from structural studies of beta-catenin and its binding partners., J. Cell Sci. 120 (2007) 3337–44.

[45] M. Johnson, M. Sharma, C. Jamieson, J.M. Henderson, M.T.S. Mok, L. Bendall, B.R. Henderson, Regulation of β-catenin trafficking to the membrane in living cells, Cell. Signal. 21 (2009) 339–348.

[46] Y. Komiya, R. Habas, Wnt signal transduction pathways., Organogenesis. 4 (2008) 68–75.

[47] C. Niehrs, S.P. Acebron, Mitotic and mitogenic Wnt signalling, EMBO J. 31 (2012) 2705–2713.

[48] C. Niehrs, The complex world of WNT receptor signalling., Nat. Rev. Mol. Cell Biol. 13 (2012) 767–779.

[49] S.P. Acebron, E. Karaulanov, B.S. Berger, Y.L. Huang, C. Niehrs, Mitotic Wnt Signaling Promotes Protein Stabilization and Regulates Cell Size, Mol. Cell. 54 (2014) 663–674.

[50] D.S. Micalizzi, S.M. Farabaugh, H.L. Ford, Epithelial-mesenchymal transition in cancer: Parallels between normal development and tumor progression, J. Mammary Gland Biol. Neoplasia. 15 (2010) 117–134.

[51] A.H. Huber, W.J. Nelson, W.I. Weis, Three-dimensional structure of the armadillo repeat region of beta-catenin., Cell. 90 (1997) 871–82.

[52] Y. Xing, K.-I. Takemaru, J. Liu, J.D. Berndt, J.J. Zheng, R.T. Moon, W. Xu, Crystal structure of a full-length beta-catenin., Structure. 16 (2008) 478–487.

[53] T.A. Graham, D.M. Ferkey, F. Mao, D. Kimelman, W. Xu, Tcf4 can specifically recognize beta-catenin using alternative conformations., Nat. Struct. Biol. 8 (2001) 1048–52.

[54] T.A. Graham, W.K. Clements, D. Kimelman, W. Xu, The crystal structure of the beta-catenin/ICAT complex reveals the inhibitory mechanism of ICAT., Mol. Cell. 10 (2002) 563–71.

[55] T.A. Graham, C. Weaver, F. Mao, D. Kimelman, W. Xu, Crystal structure of a beta-catenin/Tcf complex., Cell. 103 (2000) 885–896.

[56] F. Poy, M. Lepourcelet, R.A. Shivdasani, M.J. Eck, Structure of a human Tcf4-beta-catenin complex., Nat. Struct. Biol. 8 (2001) 1053–7.

[57] F. Fagotto, Looking beyond the Wnt pathway for the deep nature of β-catenin., EMBO Rep. 14 (2013) 422–33.

[58] F. Fagotto, U. Glück, B.M. Gumbiner, Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of beta-catenin., Curr. Biol. 8 (1998) 181–190.

[59] C. Jamieson, M. Sharma, B.R. Henderson, Targeting the β-catenin nuclear transport pathway in cancer, Semin. Cancer Biol. 27 (2014) 20–29.

[60] H.-J. Choi, A.H. Huber, W.I. Weis, Thermodynamics of beta-catenin-ligand interactions: the roles of the N- and C-terminal tails in modulating binding affinity., J. Biol. Chem. 281 (2006) 1027–38.

[61] S. Knapp, M. Zamai, D. Volpi, V. Nardese, N. Avanzi, J. Breton, S. Plyte, M. Flocco, M. Marconi, A. Isacchi, V.R. Caiolfa, Thermodynamics of the high-affinity interaction of TCF4 with beta-catenin., J. Mol. Biol. 306 (2001) 1179–1189.

[62] B.R. Henderson, M. Galea, S. Schuechner, L. Leung, Lymphoid enhancer factor-1 blocks adenomatous polyposis coli-mediated nuclear export and degradation of beta-catenin. Regulation by histone deacetylase 1., J. Biol. Chem. 277 (2002) 24258–64.

[63] L. Arce, N.N. Yokoyama, M.L. Waterman, Diversity of LEF/TCF action in development and disease., Oncogene. 25 (2006) 7492–7504.

[64] S. Hoppler, C.L. Kavanagh, Wnt signalling : variety at the core, (2007) 385–393.

[65] R. Mishra, Glycogen synthase kinase 3 beta: can it be a target for oral cancer., Mol. Cancer. 9 (2010) 144.

[66] T.D. King, Y. Li, Dysregulation of Wnt Signaling in Breast Cancer, 5 (2009).

[67] K.M. Cadigan, M.L. Waterman, TCF / LEFs and Wnt Signaling in the Nucleus, Cold Spring Harb Symp Quant Biol. 4 (2012) 1–22.

[68] P.J. Morin, A.B. Sparks, V. Korinek, N. Barker, H. Clevers, B. Vogelstein, K.W. Kinzler, Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC., Science (80) 275 (1997) 1787–1790.

[69] B. Rubinfeld, Stabilization of beta-catenin by genetic defects in melanoma cell lines., Science (80) 275 (1997) 1790–1792.

[70] M. Hart, J.P. Concordet, I. Lassot, I. Albert, R. Del Los Santos, H. Durand, C. Perret, B. Rubinfeld, F. Margottin, R. Benarous, P. Polakis, The F-box protein b-TrCP associates with phosphorylated b-catenin and regulates its activity in the cell, Curr. Biol. 9 (1999) 207–210.

[71] A. de La Coste, B. Romagnolo, P. Billuart, C.-A.A. Renard, M.-A.A. Buendia, O. Soubrane, M. Fabre, J. Chelly, C. Beldjord, A. Kahn, C. Perret, A. d. L. Coste, B. Romagnolo, P. Billuart, C.-A.A. Renard, M.-A.A. Buendia, O. Soubrane, M. Fabre, J. Chelly, C. Beldjord, A. Kahn, C. Perret, Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas., Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 8847–8851.

[72] Y. Miyoshi, K. Iwao, Y. Nagasawa, T. Aihara, Y. Sasaki, S. Imaoka, M. Murata, T. Shimano, Y. Nakamura, Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3., Cancer Res.

58 (1998) 2524–7.

[73] H. Huang, H. Fujii, A. Sankila, B.M. Mahler-Araujo, M. Matsuda, G. Cathomas, H. Ohgaki, Beta-catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C virus infection., Am. J. Pathol. 155 (1999) 1795–801.

[74] K. Shitoh, F. Konishi, T. Iijima, T. Ohdaira, K. Sakai, K. Kanazawa, M. Miyaki, A novel case of a sporadic desmoid tumour with mutation of the beta catenin gene, J Clin Pathol. 52 (1999) 695–696.

[75] S. Tejpar, F. Nollet, C. Li, J.S. Wunder, G. Michils, P. dal Cin, E. Van Cutsem, B. Bapat, F. van Roy, J.J. Cassiman, B.A. Alman, Predominance of beta-catenin mutations and beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor)., Oncogene. 18 (1999) 6615–6620.

[76] Y. Wei, M. Fabre, S. Branchereau, F. Gauthier, G. Perilongo, M.A. Buendia, Activation of beta-catenin in epithelial and mesenchymal hepatoblastomas., Oncogene. 19 (2000) 498–504.

[77] A. Koch, D. Denkhaus, S. Albrecht, I. Leuschner, D. von Schweinitz, T. Pietsch, Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene., Cancer Res. 59 (1999) 269–273.

[78] J. Palacios, C. Gamallo, Mutations in the beta-catenin gene (CTNNB1) in endometrioid ovarian carcinomas., Cancer Res. 58 (1998) 1344–1347.

[79] C. Gamallo, J. Palacios, G. Moreno, J. Calvo de Mora, A. Suarez, A. Armas, beta-catenin expression pattern in stage I and II ovarian carcinomas : relationship with beta-catenin gene mutations, clinicopathological features, and clinical outcome., Am. J. Pathol. 155 (1999) 527–536.

[80] K. Wright, P. Wilson, S. Morland, I. Campbell, M. Walsh, T. Hurst, B. Ward, M. Cummings, G. Chenevix-Trench, Beta-Catenin Mutation and Expression Analysis in Ovarian Cancer: Exon 3 Mutations and Nuclear Translocation in 16% of Endometrioid Tumours., Int. J. Cancer. 82 (1999) 625–629.

[81] K. Iwao, S. Nakamori, M. Kameyama, S. Imaoka, M. Kinoshita, T. Fukui, S. Ishiguro, Y. Nakamura, Y. Miyoshi, Activation of the b-Catenin Gene by Interstitial Deletions Involving Exon 3 in Primary Colorectal Carcinomas without Adenomatous Polyposis Coli Mutations1 ExonS ExonS Normal Transcript Deleted Transcript, Cancer Res. (1998) 1021–1026.

[82] A.B. Sparks, P.J. Morin, B. Vogelstein, K.W. Kinzler, Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer., Cancer Res. 58 (1998) 1130–1134.

[83] M. Ni, Y. Chen, E. Lim, H. Wimberly, S.T. Bailey, Y. Imai, D.L. Rimm, X. Shirley Liu, M. Brown, Targeting Androgen Receptor in Estrogen Receptor-Negative Breast Cancer, Cancer Cell. 20 (2011) 119–131.

[84] T. Hu, C. Li, Convergence between Wnt-β-catenin and EGFR signaling in cancer., Mol. Cancer. 9 (2010) 236.

[85] Z.F. Zimmerman, R.T. Moon, A.J. Chien, Targeting Wnt pathways in disease, Cold Spring Harb. Perspect. Biol. 4 (2012).

[86] R. Thakur, D.P. Mishra, Pharmacological modulation of beta-catenin and its applications in cancer therapy., J. Cell. Mol. Med. 17 (2013) 449–56.

[87] A. Saadeddin, R. Babaei-Jadidi, B. Spencer-Dene, A.S. Nateri, The links between transcription, beta-catenin/JNK signaling, and carcinogenesis., Mol. Cancer Res. 7 (2009) 1189–1196.

[88] C.A. Thorne, A.J. Hanson, J. Schneider, E. Tahinci, D. Orton, C.S. Cselenyi, K.K. Jernigan, K.C. Meyers, B.I. Hang, A.G. Waterson, K. Kim, B. Melancon, V.P. Ghidu, G.A. Sulikowski, B. LaFleur, A. Salic, L.A. Lee, D.M. Miller, E. Lee, Small-molecule inhibition of Wnt signaling through activation of casein kinase 1α, Nat Chem Biol. 6 (2010) 829–836..

[89] S.-M.A. Huang, Y.M. Mishina, S. Liu, A. Cheung, F. Stegmeier, G.A. Michaud, O. Charlat, E. Wiellette, Y. Zhang, S. Wiessner, M. Hild, X. Shi, C.J. Wilson, C. Mickanin, V. Myer, A. Fazal, R. Tomlinson, F. Serluca, W. Shao, H. Cheng, M. Shultz, C. Rau, M. Schirle, J. Schlegl, S. Ghidelli, S. Fawell, C. Lu, D. Curtis, M.W. Kirschner, C. Lengauer, P.M. Finan, J.A. Tallarico, T. Bouwmeester, J.A. Porter, A. Bauer, F. Cong, Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling, Nature. 461 (2009) 614–620.

[90] M. Lepourcelet, Y.-N.P. Chen, D.S. France, H. Wang, P. Crews, F. Petersen, C. Bruseo, A.W. Wood, R.A. Shivdasani, Small-molecule antagonists of the oncogenic Tcf/beta-catenin protein complex., Cancer Cell. 5 (2004) 91–102.

[91] K.H. Emami, C. Nguyen, H. Ma, D.H. Kim, K.W. Jeong, M. Eguchi, R.T. Moon, J.-L. Teo, H.Y. Kim, S.H. Moon, J.R. Ha, M. Kahn, A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected]., Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 12682–12687.

[92] J. Shan, D.-L. Shi, J. Wang, J. Zheng, Identification of a specific inhibitor of the dishevelled PDZ domain., Biochemistry. 44 (2005) 15495–15503.

[93] Z. Chen, A.M. Venkatesan, C.M. Dehnhardt, O. Dos Santos, E. Delos Santos, S. Ayral-Kaloustian, L. Chen, Y. Geng, K.T. Arndt, J. Lucas, I. Chaudhary, T.S. Mansour, 2,4-Diamino-quinazolines as inhibitors of beta-catenin/Tcf-4 pathway: Potential treatment for colorectal cancer., Bioorg. Med. Chem. Lett. 19 (2009) 4980–4983. 0.

[94] C.H. Park, E.R. Hahm, S. Park, H.-K. Kim, C.H. Yang, The inhibitory mechanism of curcumin and its derivative against beta-catenin/Tcf signaling., FEBS Lett. 579 (2005) 2965–2971. [95] C.H. Park, J.Y. Chang, E.R. Hahm, S. Park, H.-K. Kim, C.H. Yang, Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells., Biochem. Biophys. Res. Commun. 328 (2005) 227–234.

[96] K. Yang, X. Wang, H. Zhang, Z. Wang, G. Nan, Y. Li, F. Zhang, M.K. Mohammed, R.C. Haydon, H.H. Luu, Y. Bi, T.-C. He, The evolving roles of canonical WNT signaling in stem cells and tumorigenesis: implications in targeted cancer therapies, Lab. Investig. 96 (2016) 1–21.

[97] A. Blagodatski, D. Poteryaev, V.L. Katanaev, Targeting the Wnt pathways for therapies, Mol. Cell. Ther. 2 (2014) 28.

[98] D.C. Smith, M. Gordon, W. Messersmith, R. Chugh, D. Mendelson, J. Dupont, R. Stagg, A.M. Kapoun, L.L. Xu, R.K. Brachmann, A. Jimeno, A first-in-human Phase 1 study of anti-cancer stem cell (CSC) agent OMP-54F28 (FZD8-Fc) targeting the WNT pathway in patients with advanced solid tumors., Mol. Cancer Ther. 12 (2014) B79 LP-B79.

[99] B. Chen, M.E. Dodge, W. Tang, J. Lu, Z. Ma, C.-W. Fan, S. Wei, W. Hao, J. Kilgore, N.S. Williams, M.G. Roth, J.F. Amatruda, C. Chen, L. Lum, Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer., Nat. Chem. Biol. 5 (2009) 100–107.

[100] T. Lau, E. Chan, M. Callow, J. Waaler, J. Boggs, R.A. Blake, S. Magnuson, A. Sambrone, M. Schutten, R. Firestein, O. Machon, V. Korinek, E. Choo, D. Diaz, M. Merchant, P. Polakis, D.D. Holsworth, S. Krauss, M. Costa, A novel tankyrase small-molecule inhibitor suppresses APC mutation-driven colorectal tumor growth, Cancer Res. 73 (2013) 3132–3144.

[101] J. Waaler, O. Machon, L. Tumova, H. Dinh, V. Korinek, S.R. Wilson, J.E. Paulsen, N.M. Pedersen, T.J. Eide, O. Machonova, D. Gradl, A. Voronkov, J.P. Von Kries, S. Krauss, A novel tankyrase inhibitor decreases canonical Wnt signaling in colon carcinoma cells and reduces tumor growth in conditional APC mutant mice, Cancer Res. 72 (2012) 2822–2832.

[102] F.C. Gonsalves, K. Klein, B.B. Carson, S. Katz, L.A. Ekas, S. Evans, R. Nagourney, T. Cardozo, A.M.C. Brown, R. DasGupta, An RNAi-based chemical genetic screen identifies three small-molecule inhibitors of the Wnt/wingless signaling pathway., Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 5954–5963.

[103] J.-Y. Trosset, C. Dalvit, S. Knapp, M. Fasolini, M. Veronesi, S. Mantegani, L.M. Gianellini, C. Catana, M. Sundstrom, P.F.W. Stouten, J.K. Moll, Inhibition of protein-protein interactions: the discovery of druglike beta-catenin inhibitors by combining virtual and biophysical screening., Proteins. 64 (2006) 60–67.

[104] W. Tian, X. Han, M. Yan, Y. Xu, S. Duggineni, N. Lin, G. Luo, Y.M. Li, X. Han, Z. Huang, J. An, Structure-based discovery of a novel inhibitor targeting the beta-catenin/Tcf4 interaction., Biochemistry. 51 (2012) 724–731.

[105] H. Yao, E. Ashihara, J.W. Strovel, Y. Nakagawa, J. Kuroda, R. Nagao, R. Tanaka, A. Yokota, M. Takeuchi, Y. Hayashi, C. Shimazaki, M. Taniwaki, K. Strand, J. Padia, H. Hirai, S. Kimura, T. Maekawa, AV-65, a novel Wnt/β-catenin signal inhibitor, successfully suppresses progression of multiple myeloma in a mouse model., Blood Cancer J. 1 (2011) e43.

[106] T.N. Grossmann, J.T.-H. Yeh, B.R. Bowman, Q. Chu, R.E. Moellering, G.L. Verdine, Inhibition of Oncogenic Wnt Signaling Through Direct Targeting of β-catenin., Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 17942–7.

[107] C.M. Snow, A. Senior, L. Gerace, Monoclonal antibodies identify a group of nuclear pore complex glycoproteins, J. Cell Biol. 104 (1987) 1143–1156.

[108] J.Z. Gasiorowski, D.A. Dean, Mechanisms of nuclear transport and interventions, Adv. Drug Deliv. Rev. 55 (2003) 703–716.

[109] S. Kosugi, M. Hasebe, T. Entani, S. Takayama, M. Tomita, H. Yanagawa, Design of Peptide Inhibitors for the Importin α/β Nuclear Import Pathway by Activity-Based Profiling, Chem. Biol. 15 (2008) 940–949.

[110] H.-J. Lenz, M. Kahn, Safely targeting cancer stem cells via selective catenin coactivator antagonism., Cancer Sci. 105 (2014) 1087–1092.

[111] N. Baryawno, B. Sveinbjornsson, S. Eksborg, C.-S. Chen, P. Kogner, J.I. Johnsen, Small-molecule inhibitors of phosphatidylinositol 3-kinase/Akt signaling inhibit Wnt/beta-catenin pathway cross-talk and suppress medulloblastoma growth., Cancer Res. 70 (2010) 266–276.

[112] I. Collins, P. Workman, New approaches to molecular cancer therapeutics., Nat. Chem. Biol. 2 (2006) 689–700.

[113] J.G. Christensen, R. Schreck, J. Burrows, P. Kuruganti, E. Chan, P. Le, J. Chen, X. Wang, L. Ruslim, R. Blake, K.E. Lipson, J. Ramphal, S. Do, J.J. Cui, J.M. Cherrington, D.B. Mendel, A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes in vitro and exhibits cytoreductive antitumor activity in vivo., Cancer Res. 63 (2003) 7345–7355.

[114] T. Osada, M. Chen, X.Y. Yang, I. Spasojevic, J.B. Vandeusen, D. Hsu, B.M. Clary, T.M. Clay, W. Chen, M.A. Morse, H.K. Lyerly, Antihelminth compound niclosamide downregulates Wnt signaling and elicits antitumor responses in tumors with activating APC mutations., Cancer Res. 71 (2011) 4172–4182.

[115] M. Chen, J. Wang, J. Lu, M.C. Bond, X.-R. Ren, H.K. Lyerly, L.S. Barak, W. Chen, The anti-helminthic niclosamide inhibits Wnt/Frizzled1 signaling., Biochemistry. 48 (2009) 10267–10274.

[116] W. Lu, C. Lin, T.D. King, H. Chen, R.C. Reynolds, Y. Li, Silibinin Inhibits Wnt/β-catenin Signaling by Suppressing Wnt Co-receptor LRP6 Expression in Human Prostate and Breast Cancer Cells., Cell. Signal. 24 (2012) 2291–6.

[117] W. Lu, C. Lin, Y. Li, Rottlerin induces Wnt co-receptor LRP6 degradation and suppresses both Wnt/beta-catenin and mTORC1 signaling in prostate and breast cancer cells., Cell. Signal. 26 (2014) 1303–1309.

[118] H.S. Rahman, A. Rasedee, S.K. Yeap, H.H. Othman, M.S. Chartrand, F. Namvar, A.B. Abdul, C.W. How, Biomedical properties of a natural dietary plant metabolite, Zerumbone, in cancer therapy and chemoprevention trials, Biomed Res. Int. 2014 (2014).

[119] S.I. Abdelwahab, A.B. Abdul, S. Mohan, M.M.E. Taha, S. Syam, M.Y. Ibrahim, A.A. Mariod, Zerumbone induces apoptosis in T-acute lymphoblastic leukemia cells., Leuk. Res. 35 (2011) 268–271.

[120] S.A.S. Sakinah, S.T. Handayani, L.P.A. Hawariah, Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio., Cancer Cell Int. 7 (2007) 1–11.

[121] S.I. Abdelwahab, A.B. Abdul, Z.N.M. Zain, A.H.A. Hadi, Zerumbone inhibits interleukin-6 and induces apoptosis and cell cycle arrest in ovarian and cervical cancer cells., Int. Immunopharmacol. 12 (2012) 594–602.

[122] N.J. Yob, S.M. Jofrry, M.M.R.M.M. Affandi, L.K. Teh, M.Z. Salleh, Z. a Zakaria, Zingiber zerumbet (L.) Smith: A Review of Its Ethnomedicinal, Chemical, and Pharmacological Uses., Evid. Based. Complement. Alternat. Med. 2011 (2011) 543216.

[123] A.Y. Koga, F.L. Beltrame, A. V. Pereira, Several aspects of Zingiber zerumbet: a review, Rev. Bras. Farmacogn. 26 (2016) 385–391.

[124] J. Dai, J.H. Cardellina, J.B.M. Mahon, M.R. Boyd, Zerumbone, an HIV-Inhibitory and Cytotoxic Sesquiterpene of Zingiber Aromaticum and Z. Zerumbet, Nat. Prod. Lett. 10 (1997) 115–118.

[125] A. Murakami, T. Shigemori, H. Ohigashi, Zingiberaceous and citrus constituents, 1'-acetoxychavicol acetate, zerumbone, auraptene, and nobiletin, suppress lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264. 7 murine macrophages through different modes of action, J. Nutr. (2005) 2987–2992.

[126] M.M.E. Taha, A.B. Abdul, R. Abdullah, T.A.T. Ibrahim, S.I. Abdelwahab, S. Mohan, Potential chemoprevention of diethylnitrosamine-initiated and 2-acetylaminofluorene-promoted hepatocarcinogenesis by zerumbone from the rhizomes of the subtropical ginger (Zingiber zerumbet)., Chem. Biol. Interact. 186 (2010) 295–305.

[127] A. Murakami, T. Tanaka, J. Lee, Zerumbone, a sesquiterpene in subtropical ginger, suppresses skin tumor initiation and promotion stages in ICR mice,.J. Cancer. 110 (2004) 481–90.

[128] A. Hoffman, L.M. Spetner, M. Burke, Redox-regulated mechanism may account for zerumbone's ability to suppress cancer-cell proliferation., Carcinogenesis. 23 (2002) 1961.

[129] A. Deorukhkar, N. Ahuja, A.L. Mercado, P. Diagaradjane, U. Raju, N. Patel, P. Mohindra, N. Diep, S. Guha, S. Krishnan, Zerumbone increases oxidative stress in a thiol-dependent ROS-independent manner to increase DNA damage and sensitize colorectal cancer cells to radiation, Cancer Med. 4 (2015) 278–292.

[130] S.-H. Choi, Y.-J. Lee, W.D. Seo, H.-J. Lee, J.-W. Nam, Y.J. Lee, J. Kim, E.-K. Seo, Y.-S. Lee, Altered cross-linking of HSP27 by zerumbone as a novel strategy for overcoming HSP27-mediated radioresistance., Int. J. Radiat. Oncol. Biol.

Phys. 79 (2011) 1196–1205.

[131] A. Murakami, R. Hayashi, T. Tanaka, K.H. Kwon, H. Ohigashi, R. Safitri, Suppression of dextran sodium sulfate-induced colitis in mice by zerumbone, a subtropical ginger sesquiterpene, and nimesulide: separately and in combination., Biochem. Pharmacol. 66 (2003) 1253–1261.

[132] Y. Igarashi, K. Ohnishi, K. Irie, A. Murakami, Possible Contribution of Zerumbone-Induced Proteo-Stress to Its Anti-Inflammatory Functions via the Activation of Heat Shock Factor 1., PLoS One. 11 (2016) e0161282.

[133] ChemSpider, Zerumbone Structure, (n.d.). http://www.chemspider.com/Chemical-Structure.4580581.html.

[134] R. Baron, J.A. McCammon, Molecular recognition and ligand association., Annu. Rev. Phys. Chem. 64 (2013) 151–75.

[135] Y. Tang, Examination of Molecular Recognition in Protein-Ligand Interactions, 2010.

[136] M.K. Haider, Computational Analyses of Protein-ligand Interaction, 2010. http://etheses.whiterose.ac.uk/1242/2/thesis_final_mkh_updated.pdf.

[137] A. Nicholls, K.A. Sharp, B. Honig, Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons., Proteins. 11 (1991) 281–296.

[138] C. Bissantz, B. Kuhn, M. Stahl, A medicinal chemist's guide to molecular interactions, J. Med. Chem. 53 (2010) 5061–5084.

[139] H. Gohlke, G. Klebe, Approaches to the description and prediction of the binding affinity of small-molecule ligands to macromolecular receptors., Angew. Chem. Int. Ed. Engl. 41 (2002) 2644–2676.

[140] C. Liu, S.T. Wrobleski, J. Lin, G. Ahmed, A. Metzger, J. Wityak, K.M. Gillooly, D.J. Shuster, K.W. McIntyre, S. Pitt, D.R. Shen, R.F. Zhang, H. Zhang, A.M. Doweyko, D. Diller, I. Henderson, J.C. Barrish, J.H. Dodd, G.L. Schieven, K. Leftheris, 5-Cyanopyrimidine derivatives as a novel class of potent, selective, and orally active inhibitors of p38alpha MAP kinase., J. Med. Chem. 48 (2005) 6261–6270.

[141] C.A. Chang, W. Chen, M.K. Gilson, Ligand configurational entropy and protein binding., Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 1534–1539.

[142] I. Massova, P.A. Kollman, Combined molecular mechanical and continuum solvent approach (MM-PBSA/GBSA) to predict ligand binding, Perspect. Drug Discov. Des. 18 (2000) 113–135.

[143] T. Lazaridis, Inhomogeneous Fluid Approach to Solvation Thermodynamics. 1. Theory, J. Phys. Chem. B. 102 (1998) 3531–3541.

[144] D.J. Huggins, M.C. Payne, Assessing the Accuracy of Inhomogeneous Fluid Solvation Theory in Predicting Hydration Free Energies of Simple Solutes, J.

Phys. Chem. B. 117 (2013) 8232–8244.

[145] W.L. Jorgensen, Drug discovery: Pulled from a protein's embrace, Nature. 466 (2010) 42–43.

[146] G. Sliwoski, S. Kothiwale, J. Meiler, E.W. Lowe, Computational Methods in Drug Discovery, (2014) 334–395.

[147] M. Pellecchia, I. Bertini, D. Cowburn, C. Dalvit, E. Giralt, W. Jahnke, T.L. James, S.W. Homans, H. Kessler, C. Luchinat, B. Meyer, H. Oschkinat, J. Peng, H. Schwalbe, G. Siegal, Perspectives on NMR in drug discovery: a technique comes of age, Nat. Rev. Drug Discov. 7 (2008) 738–745.

[148] F.C. Bernstein, T.F. Koetzle, G.J. Williams, E.F. Meyer, M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi, M. Tasumi, The Protein Data Bank, Eur. J. Biochem. 324 (1977) 319–324.

[149] V. Corradi, M. Mancini, M.A. Santucci, T. Carlomagno, D. Sanfelice, M. Mori, G. Vignaroli, F. Falchi, F. Manetti, M. Radi, M. Botta, Computational techniques are valuable tools for the discovery of protein-protein interaction inhibitors: the 14-3-3σ case., Bioorg. Med. Chem. Lett. 21 (2011) 6867–6871.

[150] N. Eswar, B. Webb, M.A. Marti-Renom, M.S. Madhusudhan, D. Eramian, M. Shen, U. Pieper, A. Sali, Comparative protein structure modeling using Modeller, Curr. Protoc. Protein Sci. Chapter 2 (2006) Unit 2.9.

[151] R. A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, PROCHECK: a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283–291.

[152] G. Vriend, WHAT IF: A molecular modeling and drug design program, J. Mol. Graph. 8 (1990) 52–56.

[153] T. Schwede, SWISS-MODEL: an automated protein homology-modeling server, Nucleic Acids Res. 31 (2003) 3381–3385.

[154] U. Pieper, B.M. Webb, G.Q. Dong, D. Schneidman-Duhovny, H. Fan, S.J. Kim, N. Khuri, Y.G. Spill, P. Weinkam, M. Hammel, J.A. Tainer, M. Nilges, A. Sali, ModBase, a database of annotated comparative protein structure models and associated resources, Nucleic Acids Res. 42 (2014) 1–11.

[155] The UniProt Consortium, UniProt: a hub for protein information, Nucleic Acids Res. 43 (2014) D204-12.

[156] H. Park, Y.J. Bahn, S.E. Ryu, Structure-based de novo design and biochemical evaluation of novel Cdc25 phosphatase inhibitors., Bioorg. Med. Chem. Lett. 19 (2009) 4330–4334.

[157] S. Henrich, O.M.H. Salo-Ahen, B. Huang, F.F. Rippmann, G. Cruciani, R.C. Wade, Computational approaches to identifying and characterizing protein binding sites for ligand design, J. Mol. Recognit. 23 (2010) 209–219.

[158] D.C. Smithson, J. Lee, A.A. Shelat, M.A. Phillips, R.K. Guy, Discovery of Potent and Selective Inhibitors of Trypanosoma brucei Ornithine Decarboxylase, J. Biol. Chem. 285 (2010) 16771–16781.

[159] A.T.R. Laurie, R.M. Jackson, Methods for the prediction of protein-ligand binding sites for structure-based drug design and virtual ligand screening., Curr. Protein Pept. Sci. 7 (2006) 395–406.

[160] A.T.R. Laurie, R.M. Jackson, Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites, Bioinformatics. 21 (2005) 1908–1916.

[161] V. Summa, A. Petrocchi, F. Bonelli, B. Crescenzi, M. Donghi, M. Ferrara, F. Fiore, C. Gardelli, O. Gonzalez Paz, D.J. Hazuda, P. Jones, O. Kinzel, R. Laufer, E. Monteagudo, E. Muraglia, E. Nizi, F. Orvieto, P. Pace, G. Pescatore, R. Scarpelli, K. Stillmock, M. V Witmer, M. Rowley, Discovery of raltegravir, a potent, selective orally bioavailable HIV-integrase inhibitor for the treatment of HIV-AIDS infection., J. Med. Chem. 51 (2008) 5843–5855.

[162] J.R. Schames, R.H. Henchman, J.S. Siegel, C.A. Sotriffer, H. Ni, J.A. McCammon, Discovery of a novel binding trench in HIV integrase., J. Med. Chem. 47 (2004) 1879–1881.

[163] Y. Zeng, R. Cao, J. Yang, X. Li, S. Li, W. Zhong, Design, synthesis and biological evaluation of novel HSP70 inhibitors: N, N′-disubstituted thiourea derivatives, Eur. J. Med. Chem. 119 (2016) 83–95.

[164] C.-H. Chuang, T.-C. Cheng, Y.-L. Leu, K.-H. Chuang, S.-C. Tzou, C.-S. Chen, Discovery of Akt Kinase Inhibitors through Structure-Based Virtual Screening and Their Evaluation as Potential Anticancer Agents, Int. J. Mol. Sci. 16 (2015) 3202–3212.

[165] I. Pauli, R.N. dos Santos, D.C. Rostirolla, L.K. Martinelli, R.G. Ducati, L.F.S.M. Timmers, L.A. Basso, D.S. Santos, R.V.C. Guido, A.D. Andricopulo, O. Norberto de Souza, Discovery of new inhibitors of Mycobacterium tuberculosis InhA enzyme using virtual screening and a 3D-pharmacophore-based approach., J. Chem. Inf. Model. 53 (2013) 2390–2401.

[166] S.Z. Grinter, X. Zou, Challenges, applications, and recent advances of protein-ligand docking in structure-based drug design, Molecules. 19 (2014) 10150–10176.

[167] S.Y. Huang, X. Zou, Advances and challenges in protein-ligand docking, Int. J. Mol. Sci. 11 (2010) 3016–3034.

[168] P. Prathipati, A. Dixit, A. Saxena, Computer-Aided Drug Design: Integration of Structure-Based and Ligand-Based Approaches in Drug Design, Curr. Comput. Aided-Drug Des. 3 (2007) 133–148.

[169] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank., Nucleic Acids Res. 28 (2000) 235–242.

[170] G.M. Morris, R. Huey, A.J. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791.

[171] G. Lemmon, J. Meiler, Rosetta Ligand docking with flexible XML protocols., Methods Mol. Biol. 819 (2012) 143–155.

[172] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings., Adv. Drug Deliv. Rev. 46 (2001) 3–26.

[173] P. Ripphausen, B. Nisius, L. Peltason, J. Bajorath, Quo vadis, virtual screening? A comprehensive survey of prospective applications., J. Med. Chem. 53 (2010) 8461–7.

[174] S. Kalyaanamoorthy, Y.-P.P. Chen, Structure-based drug design to augment hit discovery., Drug Discov. Today. 16 (2011) 831–839.

[175] R. Rohs, I. Bloch, H. Sklenar, Z. Shakked, Molecular flexibility in ab initio drug docking to DNA: binding-site and binding-mode transitions in all-atom Monte Carlo simulations., Nucleic Acids Res. 33 (2005) 7048–7057.

[176] J. Wang, P.A. Kollman, I.D. Kuntz, Flexible ligand docking: A multistep strategy approach, Proteins-Structure Funct. Genet. 36 (1999).

[177] Huang, S.Z. Grinter, X. Zou, Scoring functions and their evaluation methods for protein-ligand docking: recent advances and future directions, Phys. Chem. Chem. Phys. PCCP. 12 (2010) 12899–12908.

[178] H.V.-C. and C.M.-B. Alejandra Hernández-Santoyo, Aldo Yair Tenorio-Barajas, Victor Altuzar, Protein-Protein and Protein-Ligand Docking, Protein-Protein and Protein-Ligand Docking. (2013) 64–81.

[179] S.J. Weiner, P.A. Kollman, D.T. Nguyen, D.A. Case, An all atom force field for simulations of proteins and nucleic acids, J. Comput. Chem. 7 (1986) 230–252.

[180] S.A. Adcock, J.A. McCammon, Molecular dynamics: survey of methods for simulating the activity of proteins., Chem. Rev. 106 (2006) 1589–1615.

[181] H.F.G. Velec, H. Gohlke, G. Klebe, DrugScore(CSD)-knowledge-based scoring function derived from small molecule crystal data with superior recognition rate of near-native ligand poses and better affinity prediction., J. Med. Chem. 48 (2005) 6296–6303.

[182] W. Rocchia, S. Sridharan, A. Nicholls, E. Alexov, A. Chiabrera, B. Honig, Rapid grid-based construction of the molecular surface and the use of induced surface charge to calculate reaction field energies: applications to the molecular systems and geometric objects., J. Comput. Chem. 23 (2002) 128–137.

[183] J.A. Grant, B.T. Pickup, A. Nicholls, A smooth permittivity function for Poisson-Boltzmann solvation methods, J. Comput. Chem. 22 (2001) 608–640.

[184] J. Mitchell, R. Laskowski, A. Alex, J. Thornton, BLEEP—Potential of Mean Force Describing Protein-Ligand Interactions: I. Generating Potential, J Comp Chem. 20 (1999) 1165–1176.

[185] A. V Ishchenko, and Eugene I. Shakhnovich, SMall Molecule Growth 2001 (SMoG2001): An Improved Knowledge-Based Scoring Function for Protein−Ligand Interactions, J. Med. Chem. 45 (2002) 2770–2780.

[186] I. Muegge, Y.C. Martin, A general and fast scoring function for protein-ligand interactions: a simplified potential approach., J. Med. Chem. 42 (1999) 791–804.

[187] S.-Y. Huang, X. Zou, A knowledge-based scoring function for protein-RNA interactions derived from a statistical mechanics-based iterative method, Nucleic Acids Res. 42 (2014) e55–e55.

[188] N. Huang, C. Kalyanaraman, J.J. Irwin, M.P. Jacobson, Physics-based scoring of protein-ligand complexes: enrichment of known inhibitors in large-scale virtual screening., J. Chem. Inf. Model. 46 (2006) 243–253.

[189] D.A. Case, T.A. Darden, T.E.I. Cheatham, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, R.C. Walker, W. Zhang, K.M. Merz, B. Roberts, S. Hayik, A. Roitberg, G. Seabra, J. Swails, A.W. Goetz, I. Kolossváry, K.F. Wong, F. Paesani, J. Vanicek, J. Wolf, P.A. Kollman, AMBER 12, (2012).

[190] M. Karplus, J.A. McCammon, Molecular dynamics simulations of biomolecules., Nat. Struct. Biol. 9 (2002) 646–652.

[191] J.M. Hayes, G. Archontis, MM-GB (PB) SA Calculations of Protein-Ligand Binding Free Energies, JR Soc Interface InTech. (2011) 171–190.

[192] J. Zhu, Y. Shi, H. Liu, Parametrization of a Generalized Born/Solvent-Accessible Surface Area Model and Applications to the Simulation of Protein Dynamics, J. Phys. Chem. B. 106 (2002) 4844–4853.

[193] X. Zou, Yaxiong, I.D. Kuntz, Inclusion of Solvation in Ligand Binding Free Energy Calculations Using the Generalized-Born Model, J. Am. Chem. Soc. 121 (1999) 8033–8043.

[194] T. Hou, J. Wang, Y. Li, W. Wang, Assessing the performance of the MM/PBSA and MM/GBSA methods. 1. The accuracy of binding free energy calculations based on molecular dynamics simulations., J. Chem. Inf. Model. 51 (2011) 69–82.

[195] B.K. Fischer, High-Throughput Simulation Methods For Protein-Ligand Docking, University of Dortmund, 2007.

[196] R.A. Bryce, I.H. Hillier, Quantum Chemical Approaches : Semiempirical molecular orbital and hybrid quantum mechanical / molecular mechanical techniques ., Curr. Pharm. Des. 20 (2014) 3293–3302.

[197] A.R. Leach, Molecular Modeling: Principles and Applications, 2nd ed., Pearson, 2001.

[198] J. Meller, Molecular Dynamics, in: Encycl. Life Sci., 2001: pp. 1–8.

[199] J. Gelpi, A. Hospital, R. Goñi, M. Orozco, Molecular dynamics simulations: advances and applications, Adv. Appl. Bioinforma. Chem. (2015) 37.

[200] W. Swegat, J. Schlitter, P. Krüger, A. Wollmer, MD simulation of protein-ligand interaction: formation and dissociation of an insulin-phenol complex., Biophys. J. 84 (2003) 1493–506.

[201] A. Grover, A. Shandilya, A. Punetha, V.S. Bisaria, D. Sundar, Inhibition of the NEMO/IKKβ association complex formation, a novel mechanism associated with the NF-κB activation suppression by Withania somnifera's key metabolite withaferin A., BMC Genomics. 11 Suppl 4 (2010) S25.

[202] H. Zhao, A. Caflisch, Molecular dynamics in drug design, Eur. J. Med. Chem. 91 (2014) 4–14.

[203] H. Alonso, A. A. Bliznyuk, J.E. Gready, Combining docking and molecular dynamic simulations in drug design., Med. Res. Rev. 26 (2006) 531–68.

[204] D. Mucs, R. A. Bryce, The application of quantum mechanics in structure-based drug design., Expert Opin. Drug Discov. 8 (2013) 263–76.

[205] S. Grimme, J. Antony, S. Ehrlich, H. Krieg, A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu, J. Chem. Phys. 132 (2010).

[206] J.A. Pople, D.L. Beveridge, P.A. Dobosh, Approximate Self-Consistent Molecular-Orbital Theory. V. Intermediate Neglect of Differential Overlap, J. Chem. Phys. 47 (1967).

[207] W. Thiel, and Alexander A. Voityuk, Extension of MNDO to d Orbitals: Parameters and Results for the Second-Row Elements and for the Zinc Group, J. Phys. Chem. 100 (1996) 616–626.

[208] J.J.P. Stewart, Optimization of parameters for semiempirical methods II. Applications, J. Comput. Chem. 10 (1989) 221–264.

[209] J.J.P. Stewart, Optimization of parameters for semiempirical methods IV: Extension of MNDO, AM1 and PM3 to more main group elements, J. Mol. Model. 10 (2004) 155–164.

[210] J.J.P. Stewart, Optimization of parameters for semiempirical methods V: Modification of NDDO approximations and application to 70 elements, J. Mol. Model. 13 (2007) 1173–1213.

[211] J.J.P. Stewart, Optimization of parameters for semiempirical methods VI: More modifications to the NDDO approximations and re-optimization of parameters, J. Mol. Model. 19 (2013) 1–32.

[212] M. Korth, W. Thiel, Benchmarking semiempirical methods for thermochemistry, kinetics, and noncovalent interactions: OMx methods are almost as accurate and robust as DFT-GGA methods for organic molecules, J.

Chem. Theory Comput. 7 (2011) 2929–2936.

[213] G.B. Rocha, R.O. Freire, A.M. Simas, J.J.P. Stewart, RM1: A reparameterization of AM1 for H, C, N, O, P, S, F, Cl, Br, and I, J. Comput. Chem. 27 (2006) 1101–1111.

[214] I. Rossi, D.G. Truhlar, Parameterization of NDDO wavefunctions using genetic algorithms. An evolutionary approach to parameterizing potential energy surfaces and direct dynamics calculations for organic reactions, Chem. Phys. Lett. 233 (1995) 231–236.

[215] R. Salomon-Ferrer, D.A. Case, R.C. Walker, An overview of the Amber biomolecular simulation package, Wiley Interdiscip. Rev. Comput. Mol. Sci. 3 (2013) 198–210.

[216] K.D. Dubey, R.P. Ojha, Binding free energy calculation with QM/MM hybrid methods for Abl-Kinase inhibitor, J. Biol. Phys. 37 (2011) 69–78.

[217] F. Beierlein, H. Lanig, G. Schurer, A.H.C. Horn, Ti. Clark, Quantum mechanical/molecular mechanical (QM/MM) docking: an evaluation for known test systems, Mol. Phys. 101 (2003) 2469–2480.

[218] F. Grater, S.M. Schwarzl, A. Dejaegere, S. Fischer, J.C. Smith, Protein/ligand binding free energies calculated with quantum mechanics/molecular mechanics., J. Phys. Chem. B. 109 (2005) 10474–10483.

[219] P. Vidossich, A. Magistrato, QM/MM Molecular Dynamics Studies of Metal Binding Proteins., Biomolecules. 4 (2014) 616–45.

[220] T. Zhou, D. Huang, A. Caflisch, Quantum mechanical methods for drug design., Curr. Top. Med. Chem. 10 (2010) 33–45.

[221] A. Mulholland, Computational enzymology: modeling the mechanisms of biological catalysts, Biochem. Soc. Trans. 36 (2008) 22–6.

[222] K.D. Dubey, R.K. Tiwari, R.P. Ojha, Recent Advances in Protein Ligand Interactions : Molecular Dynamics Simulations and Binding Free Energy, Curr. Comput. Aided. Drug Des. 9 (2013).

[223] S. Izrailev, S. Stepaniants, B. Isralewitz, D. Kosztin, H. Lu, F. Molnar, W. Wriggers, K. Schulten, Steered Molecular Dynamics, Comput. Mol. Dyn. Challenges, Methods, Ideas SE - 2. 4 (1999) 39–65.

[224] E.L. Florin, V.T. Moy, H.E. Gaub, Adhesion forces between individual ligand-receptor pairs., Science. 264 (1994) 415–417.

[225] D. Van Der Spoel, E. Lindahl, B. Hess, G. Groenhof, A.E. Mark, H.J.C. Berendsen, GROMACS: fast, flexible, and free., J. Comput. Chem. 26 (2005) 1701–18.

[226] B.R. Brooks, C.L. Brooks III, A.D. Mackerell, Jr., L. Nilsson, R.J. Petrella, B. Roux, Y. Won, G. Archontis, CHARMM: The Biomolecular Simulation Program, J. Comput. Chem. 30 (2010) 1545–1614.

[227] Y. Wang, C.B. Harrison, K. Schulten, J.A. McCammon, Implementation of accelerated molecular dynamics in NAMD, Comput. Sci. Discov. 4 (2011) 15002.

[228] D.F. Gauto, C. Modenutti, V.G. Dumas, L. Alvarez, J.P. Bustamante, A.G. Turjanski, M.A. Marti, Determining Free Energies of Protein-Ligand Binding and Association / Dissociation Processes Using Computer Simulations, World. 1 (2012) 21–32.

[229] S. Park, F. Khalili-Araghi, E. Tajkhorshid, K. Schulten, Free energy calculation from steered molecular dynamics simulations using Jarzynski's equality, J. Chem. Phys. 119 (2003) 3559.

[230] M. Suan Li, B. Khanh Mai, Steered molecular dynamics-A promising tool for drug design, Curr. Bioinform. 7 (2012) 342–351.

[231] B.K. Mai, M.H. Viet, M.S. Li, Top leads for swine influenza A/H1N1 virus revealed by steered molecular dynamics approach., J. Chem. Inf. Model. 50 (2010) 2236–2247.

[232] B.K. Mai, M.S. Li, Neuraminidase inhibitor R-125489 – A promising drug for treating influenza virus: Steered molecular dynamics approach, Biochem. Biophys. Res. Commun. 410 (2011) 688–691.

[233] H. Grubmuller, B. Heymann, P. Tavan, Ligand binding: molecular mechanics calculation of the streptavidin-biotin rupture force., Science. 271 (1996) 997–999.

[234] B. Isralewitz, S. Izrailev, K. Schulten, Binding pathway of retinal to bacterio-opsin: a prediction by molecular dynamics simulations., Biophys. J. 73 (1997) 2972–2979.

[235] L.-J. Yang, J. Zou, H.-Z. Xie, L.-L. Li, Y.-Q. Wei, S.-Y. Yang, Steered molecular dynamics simulations reveal the likelier dissociation pathway of imatinib from its targeting kinases c-Kit and Abl., PLoS One. 4 (2009) e8470.

[236] X.-J. Xu, W.-Z. Chen, C.-X. Wang, S. Cannisatro, A.-R. Bizzarri, Steered Molecular Dynamics of an anticancer peptide interacting with the p53 DNA-Binding, Prog. Biochem. Biophys. (2013) 1–15.

[237] J. Gu, H. Li, X. Wang, A self-adaptive steered molecular dynamics method based on minimization of stretching force reveals the binding affinity of protein-ligand complexes, Molecules. 20 (2015) 19236–19251.

[238] K.M. Mills, Potentials of Mean Force as a Starting Point for Understanding Biomolecular Interactions by, Univ. Michigan. (2010) 209.

[239] D. Plouffe, A. Brinker, C. McNamara, K. Henson, N. Kato, K. Kuhen, A. Nagle, F. Adrián, J.T. Matzen, P. Anderson, T.-G. Nam, N.S. Gray, A. Chatterjee, J. Janes, S.F. Yan, R. Trager, J.S. Caldwell, P.G. Schultz, Y. Zhou, E. a Winzeler, In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen., Proc. Natl. Acad. Sci. U. S. A. 105

(2008) 9059–9064.

[240] A. Grover, A. Shandilya, V.S. Bisaria, D. Sundar, Probing the anticancer mechanism of prospective herbal drug Withaferin A on mammals: a case study on human and bovine proteasomes., BMC Genomics. 11 Suppl 4 (2010) S15.

[241] A. Grover, V. Agrawal, A. Shandilya, V.S. Bisaria, D. Sundar, Non-nucleosidic inhibition of Herpes simplex virus DNA polymerase: mechanistic insights into the anti-herpetic mode of action of herbal drug withaferin A., BMC Bioinformatics. 12 Suppl 1 (2011) S22.

[242] J.-L. Zhang, Q.-C. Zheng, Z.-Q. Li, H.-X. Zhang, Molecular dynamics simulations suggest ligand's binding to nicotinamidase/pyrazinamidase., PLoS One. 7 (2012) e39546.

[243] T.T. Talele, S.A. Khedkar, A.C. Rigby, Successful applications of computer aided drug discovery: moving drugs from concept to the clinic., Curr. Top. Med. Chem. 10 (2010) 127–41.

[244] G. Xu, Y.-C. Lo, Q. Li, G. Napolitano, X. Wu, X. Jiang, M. Dreano, M. Karin, H. Wu, Crystal structure of inhibitor of κB kinase β., Nature. 472 (2011) 325–330.

[245] M. Vieth, J.D. Hirst, A. Kolinski, C.L. Brooks, Assessing energy functions for flexible docking, J. Comput. Chem. 19 (1998) 1612–1622.

[246] A.D. MacKerell, D. Bashford, M. Bellott, R.L. Dunbrack, J.D. Evanseck, M.J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F.T.K. Lau, C. Mattos, S. Michnick, T. Ngo, D.T. Nguyen, B. Prodhom, W.E. Reiher, B. Roux, M. Schlenkrich, J.C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, M. Karplus, All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins, J. Phys. Chem. B. 102 (1998) 3586–3616.

[247] G. Wu, D.H. Robertson, C.L. Brooks, M. Vieth, Detailed analysis of grid-based molecular docking: A case study of CDOCKER-A CHARMm-based MD docking algorithm., J. Comput. Chem. 24 (2003) 1549–1562.

[248] M. Taufer, M. Crowley, D. Price, A.A. Chien, C.L.B. III, L. Jolla, U.S. La Jolla, Study of a Highly Accurate and Fast Protein-Ligand Docking Algorithm Based on Molecular Dynamics Center for Theoretical Biological Physics, Mol. Biol. 17 (2004) 1627–1641.

[249] F.A. Momany, R. Rone, Validation of the general purpose QUANTA ®3.2/CHARMm® force field, J. Comput. Chem. 13 (1992) 888–900.

[250] M.P. van Iersel, T. Kelder, A.R. Pico, K. Hanspers, S. Coort, B.R. Conklin, C. Evelo, Presenting and exploring biological pathways with PathVisio., BMC Bioinformatics. 9 (2008) 399.

[251] D. Seeliger, B.L. de Groot, Ligand docking and binding site analysis with PyMOL and Autodock/Vina., J. Comput. Aided. Mol. Des. 24 (2010) 417–422.

[252] K. Tago, T. Nakamura, M. Nishita, J. Hyodo, S. Nagai, Y. Murata, S. Adachi, S. Ohwada, Y. Morishita, H. Shibuya, T. Akiyama, Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein., Genes Dev. 14 (2000) 1741–1749.

[253] M.M. He, A.S. Smith, J.D. Oslob, W.M. Flanagan, A.C. Braisted, A. Whitty, M.T. Cancilla, J. Wang, A. a Lugovskoy, J.C. Yoburn, A.D. Fung, G. Farrington, J.K. Eldredge, E.S. Day, L. a Cruz, T.G. Cachero, S.K. Miller, J.E. Friedman, I.C. Choong, B.C. Cunningham, Small-molecule inhibition of TNF-alpha., Science. 310 (2005) 1022–5.

[254] M.A. Rivas, R.P. Carnevale, C.J. Proietti, C. Rosemblit, W. Beguelin, M. Salatino, E.H. Charreau, I. Frahm, S. Sapia, P. Brouckaert, P. V Elizalde, R. Schillaci, TNF alpha acting on TNFR1 promotes breast cancer growth via p42/P44 MAPK, JNK, Akt and NF-kappa B-dependent pathways., Exp. Cell Res. 314 (2008) 509–529..

[255] P. Vandenabeele, W. Declercq, R. Beyaert, W. Fiers, Two tumour necrosis factor receptors: structure and function., Trends Cell Biol. 5 (1995) 392–399.

[256] A. Israël, The IKK complex, a central regulator of NF-kappaB activation., Cold Spring Harb. Perspect. Biol. 2 (2010) a000158.

[257] H. Wajant, K. Pfizenmaier, P. Scheurich, Tumor necrosis factor signaling., Cell Death Differ. 10 (2003) 45–65.

[258] G.G. Xu, Y.-C. Lo, Q. Li, G. Napolitano, X. Wu, X.X. Jiang, M. Dreano, M. Karin, H. Wu, Crystal structure of inhibitor of kB kinase b (IKKb), Nature. 472 (2011) 325–30.

[259] M. Kalia, A. Kukol, Structure and dynamics of the kinase IKK-β--A key regulator of the NF-kappa B transcription factor., J. Struct. Biol. 176 (2011) 133–42.

[260] K. Nithya, N. Santhi, K. Kalaiselvi, Molecular docking of withanolides against the transcription factor nuclear factor - kappa B (NF-kB) using Glide, Adv. Biotech. 9 (2009) 23–27.

[261] M. Law, P. Corsino, N.T. Parker, B.K. Law, Identification of a small molecule inhibitor of serine 276 phosphorylation of the p65 subunit of NF-κB using in silico molecular docking, Cancer Lett. 291 (2011) 217–224.

[262] L. Piccagli, E. Fabbri, M. Borgatti, V. Bezzerri, I. Mancini, E. Nicolis, M.C. Dechecchi, I. Lampronti, G. Cabrini, R. Gambari, Docking of molecules identified in bioactive medicinal plants extracts into the p50 NF-kappaB transcription factor: correlation with inhibition of NF-kappaB/DNA interactions and inhibitory effects on IL-8 gene expression., BMC Struct. Biol. 8 (2008) 38.

[263] Y.Q. Chen, S. Ghosh, G. Ghosh, A novel DNA recognition mode by the NF-kappa B p65 homodimer., Nat. Struct. Biol. 5 (1998) 67–73.

[264] S.A.A. Fadl, Zerumbone and Small Interference RNA Induces Apoptosis in

MCF-7 Cell and Rat Mammary Gland Tumor via Inhibition of β-Catenin, University Putra Malaysia, 2013.

[265] T. Huxford, G. Ghosh, A Structural Guide to Proteins of the NF-κB Signaling Module, Cold Spring Harb. Perspect. Biol. 1 (2009) a000075.

[266] M. Rushe, L. Silvian, S. Bixler, L.L. Chen, A. Cheung, S. Bowes, H. Cuervo, S. Berkowitz, T. Zheng, K. Guckian, M. Pellegrini, A. Lugovskoy, Structure of a NEMO/IKK-associating domain reveals architecture of the interaction site., Structure. 16 (2008) 798–808.

[267] E. Sala, L. Guasch, J. Iwaszkiewicz, M. Mulero, M.-J. Salvadó, M. Pinent, V. Zoete, A. Grosdidier, S. Garcia-Vallvé, O. Michielin, G. Pujadas, Identification of human IKK-2 inhibitors of natural origin (part I): modeling of the IKK-2 kinase domain, virtual screening and activity assays., PLoS One. 6 (2011) e16903.

[268] D.-F. Lee, M.-C. Hung, Advances in targeting IKK and IKK-related kinases for cancer therapy., Clin. Cancer Res. 14 (2008) 5656–5662.

[269] J. Liddle, P. Bamborough, M.D. Barker, S. Campos, R.P.C. Cousins, G.J. Cutler, H. Hobbs, D.S. Holmes, C. Ioannou, G.W. Mellor, M.A. Morse, J.J. Payne, J.M. Pritchard, K.J. Smith, D.T. Tape, C. Whitworth, R.A. Williamson, 4-Phenyl-7-azaindoles as potent and selective IKK2 inhibitors., Bioorg. Med. Chem. Lett. 19 (2009) 2504–2508.

[270] C.M. Avila, A.B. Lopes, A.S. Gonçalves, L.L. Da Silva, N.C. Romeiro, A.L.P. Miranda, C.M.R. Sant'Anna, E.J. Barreiro, C.A.M. Fraga, Structure-based design and biological profile of (E)-N-(4-Nitrobenzylidene)-2-naphthohydrazide, a novel small molecule inhibitor of IκB kinase-β., Eur. J. Med. Chem. 46 (2011) 1245–1253.

[271] S.M. Noha, A.G. Atanasov, D. Schuster, P. Markt, N. Fakhrudin, E.H. Heiss, O. Schrammel, J.M. Rollinger, H. Stuppner, V.M. Dirsch, G. Wolber, Discovery of a novel IKK-β inhibitor by ligand-based virtual screening techniques., Bioorg. Med. Chem. Lett. 21 (2011) 577–83.

[272] H.-Y. Weng, M.-J. Hsu, C.-C. Wang, B.-C. Chen, C.-Y. Hong, M.-C. Chen, W.-T. Chiu, C.-H. Lin, Zerumbone suppresses IKKalpha, Akt, and FOXO1 activation, resulting in apoptosis of GBM 8401 cells., J. Biomed. Sci. 19 (2012) 86.

[273] A.E. Voronkov, I.I. Baskin, V.A. Palyulin, N.S. Zefirov, Molecular modeling of modified peptides, potent inhibitors of the xWNT8 and hWNT8 proteins., J. Mol. Graph. Model. 26 (2008) 1179–87.

[274] Q.U. Ain, U. Seemab, S. Rashid, M.S. Nawaz, M. A. Kamal, Prediction of structure of human WNT-CRD (FZD) complex for computational drug repurposing., PLoS One. 8 (2013) e54630.

[275] M. Shitashige, S. Hirohashi, T. Yamada, Wnt signaling inside the nucleus., Cancer Sci. 99 (2008) 631–637.

[276] J. Behari, The Wnt/β-catenin signaling pathway in liver biology and disease, Expert Rev. Gastroenterol. Hepatol. 4 (2010) 745–756.

[277] J. Izrailit, M. Reedijk, Developmental pathways in breast cancer and breast tumor-initiating cells: therapeutic implications., Cancer Lett. 317 (2012) 115–126.

[278] H.H. Luu, R. Zhang, R.C. Haydon, E. Rayburn, Q. Kang, W. Si, J.K. Park, H. Wang, Y. Peng, W. Jiang, T. He, β-Catenin Signaling Pathway as Novel Cancer Drug Targets Wnt / β, Curr. Cancer Drug Targets. 4 (2004) 653–671.

[279] L.M. Heiser, A. Sadanandam, W. Kuo, S.C. Benz, T.C. Goldstein, S. Ng, W.J. Gibb, N.J. Wang, F. Tong, N. Bayani, Z. Hu, J.I. Billig, A. Dueregger, S. Lewis, L. Jakkula, J.E. Korkola, S. Durinck, F. Pepin, Y. Guan, E. Purdom, P. Neuvial, H. Bengtsson, K.W. Wood, P.G. Smith, L.T. Vassilev, B.T. Hennessy, J. Greshock, K.E. Bachman, M. Ann, J.W. Park, L.J. Marton, D.M. Wolf, E.A. Collisson, R.M. Neve, G.B. Mills, T.P. Speed, H.S. Feiler, R.F. Wooster, D. Haussler, J.M. Stuart, J.W. Gray, P.T. Spellman, Subtype and pathway specific responses to anticancer compounds in breast cancer, Proc. Natl. Acad. Sci. 109 (2011) 2–7.

[280] A.M. Many, A.M.C. Brown, Mammary stem cells and cancer: roles of Wnt signaling in plain view., Breast Cancer Res. 12 (2010) 313.

[281] B. Yu, Z. Huang, M. Zhang, D.R. Dillard, H. Ji, Rational Design of Small-Molecule Inhibitors for β - Catenin/T-Cell Factor Protein − Protein Interactions by Bioisostere Replacement, ACS Chem. Biol. 8 (2013) 18–23.

[282] T. Hou, J. Wang, Y. Li, W. Wang, Assessing the performance of the MM/PBSA and MM/GBSA methods: II. The accuracy of ranking poses generated from docking, J. Comput. Chem. 32 (2011) 866–877.

[283] L. Xu, H. Sun, Y. Li, J. Wang, T. Hou, Assessing the performance of MM/PBSA and MM/GBSA methods. 3. the impact of force fields and ligand charge models, J. Phys. Chem. B. 117 (2013) 8408–8421.

[284] F.-Y. Dupradeau, A. Pigache, T. Zaffran, C. Savineau, R. Lelong, N. Grivel, D. Lelong, W. Rosanski, P. Cieplak, The R.E.D. tools: advances in RESP and ESP charge derivation and force field library building., Phys. Chem. Chem. Phys. 12 (2010) 7821–39.

[285] E. Vanquelef, S. Simon, G. Marquant, E. Garcia, G. Klimerak, J.C. Delepine, P. Cieplak, F.-Y. Dupradeau, R.E.D. Server: a web service for deriving RESP and ESP charges and building force field libraries for new molecules and molecular fragments., Nucleic Acids Res. 39 (2011) W511-7.

[286] U. Essmann, L. Perera, M.L. Berkowitz, T. Darden, H. Lee, L.G. Pedersen, A smooth particle mesh Ewald method, 103 (1995) 31–34.

[287] H. Gohlke, C. Kiel, D.A. Case, Insights into Protein–Protein Binding by Binding Free Energy Calculation and Free Energy Decomposition for the Ras–Raf and Ras–RalGDS Complexes, J. Mol. Biol. 330 (2003) 891–913.

[288] P.A. Kollman, I. Massova, C. Reyes, B. Kuhn, S. Huo, L. Chong, M. Lee, T. Lee, D.A. Case, T.E. Cheatham, Calculating Structures and Free Energies of Complex Molecules : Combining Molecular Mechanics and Continuum Models, Acc Chem. Res. 33 (2000) 889–897.

[289] M.J. Field, Paul A. Bash, M. Karplus, A combined quantum mechanical and molecular mechanical potential for molecular dynamics simulations, J. Comput. Chem. 11 (1990) 700–733.

[290] A. Warshel, M. Levitt, Theoretical studies of enzymic reactions: dielectric, electrostatic and steric stabilization of the carbonium ion in the reaction of lysozyme., J. Mol. Biol. 103 (1976) 227–249.

[291] J.P. McNamara, A.-M. Muslim, H. Abdel-Aal, H. Wang, M. Mohr, I.H. Hillier, R.A. Bryce, Towards a quantum mechanical force field for carbohydrates: a reparametrized semi-empirical MO approach, Chem. Phys. Lett. 394 (2004) 429–436.

[292] G.M. Seabra, J. Torras, R.C. Walker, A. Roitberg, U.H.E. Hansmann, J. Meinke, S. Mohanty, O.Z. Editors, G.D.M. Seabra, J. Torras, R.C. Walker, A. Roitberg, Mixed QM / MM Calculations in Biological Systems Mixed QM / MM Calculations in Biological Systems, NIC Ser. 36 (2007) 36–43.

[293] G. Lamoureux, B. Roux, Modeling induced polarization with classical Drude oscillators: Theory and molecular dynamics simulation algorithm, J. Chem. Phys. 119 (2003) 3025.

[294] A. Warshel, P.K. Sharma, M. Kato, Y. Xiang, H. Liu, M.H.M. Olsson, Electrostatic Basis for Enzyme Catalysis, Chem. Rev. 106 (2006) 3210–3235.

[295] A.K. Rappe, W.A. Goddard, Charge equilibration for molecular dynamics simulations, J. Phys. Chem. 95 (1991) 3358–3363.

[296] Y. Zhang, H. Lin, D.G. Truhlar, Self-Consistent Polarization of the Boundary in the Redistributed Charge and Dipole Scheme for Combined Quantum-Mechanical and Molecular-Mechanical Calculations, J. Chem. Theory Comput. 3 (2007) 1378–1398.

[297] L. Prof, V. Helms, W. Gu, Y. Park, Biomolecular Simulations, 924 (2013) 6–7.

[298] E. Pellegrini, M.J. Field, A Generalized-Born Solvation Model for Macromolecular Hybrid-Potential Calculations, J. Phys. Chem. A. 106 (2002) 1316–1326.

[299] http://accelrys.com/products/discovery-studio/, Improve your drug discovery process with Accelrys Discovery Studio, (n.d.). http://accelrys.com/products/discovery-studio/ (accessed October 4, 2014).

[300] K. Raha, M.B. Peters, B. Wang, N. Yu, A.M. Wollacott, L.M. Westerhoff, K.M. Merz, The role of quantum mechanics in structure-based drug design, Drug Discov. Today. 12 (2007) 725–731.

[301] N.D. Yilmazer, M. Korth, Enhanced semiempirical QM methods for biomolecular interactions, Comput. Struct. Biotechnol. J. 13 (2015) 169–175.

[302] J. Kang, Y. Hagiwara, M. Tateno, Biological applications of hybrid quantum mechanics/molecular mechanics calculation, J. Biomed. Biotechnol. 2012 (2012).

[303] A.E.D.A. Gawad, Hybrid QM/MM and classical molecular dynamics simulation of amadori product in gB-crystallin, Life Sci. J. 10 (2013) 1923–1932.

[304] J.S. Patel, A. Berteotti, S. Ronsisvalle, W. Rocchia, A. Cavalli, Steered molecular dynamics simulations for studying protein-ligand interaction in cyclin-dependent kinase 5, J. Chem. Inf. Model. 54 (2014) 470–480.

[305] M.A. Cuendet, O. Michielin, Protein-protein interaction investigated by steered molecular dynamics: the TCR-pMHC complex., Biophys. J. 95 (2008) 3575–90.

[306] J.A. Lemkul, D.R. Bevan, Destabilizing Alzheimer's Abeta(42) protofibrils with morin: mechanistic insights from molecular dynamics simulations., Biochemistry. 49 (2010) 3935–3946.

[307] K. Yang, X. Liu, X. Wang, H. Jiang, A steered molecular dynamics method with adaptive direction adjustments, Biochem. Biophys. Res. Commun. 379 (2009) 494–498.

[308] M. Musgaard, P.C. Biggin, Steered Molecular Dynamics Simulations Predict Conformational Stability of Glutamate Receptors, J. Chem. Inf. Model. 56 (2016) 1787–1797.

[309] R. Kumari, R. Kumar, A. Lynn, g_mmpbsa—A GROMACS Tool for High-Throughput MM-PBSA Calculations, J. Chem. Inf. Model. 54 (2014) 1951–1962.

[310] A.K. Malde, L. Zuo, M. Breeze, M. Stroet, D. Poger, P.C. Nair, C. Oostenbrink, A.E. Mark, An Automated Force Field Topology Builder (ATB) and Repository: Version 1.0, J. Chem. Theory Comput. 7 (2011) 4026–4037.

[311] U.C. Singh, P.A. Kollman, An approach to computing electrostatic charges for molecules, J. Comput. Chem. 5 (1984) 129–145.

[312] C. Oostenbrink, A. Villa, A.E. Mark, W.F. van Gunsteren, A biomolecular force field based on the free enthalpy of hydration and solvation: the GROMOS force-field parameter sets 53A5 and 53A6., J. Comput. Chem. 25 (2004) 1656–1676.

[313] V. Ruhle, Pressure coupling/barostats, J. Club Handout. (2008) 1–5.

[314] J.A. Lemkul, D.R. Bevan, The role of molecular simulations in the development of inhibitors of amyloid beta-peptide aggregation for the treatment of Alzheimer's disease., ACS Chem. Neurosci. 3 (2012) 845–856.

[315] C.Y. Logan, R. Nusse, the Wnt Signaling Pathway in Development and Disease, (2004).

[316] A. Wodarz, R. Nusse, Mechanisms of Wnt signalling in development., Annu. Rev. Cell Dev. Biol. 14 (1998) 59–88.

[317] U. Songsiang, S. Pitchuanchom, C. Boonyarat, C. Hahnvajanawong, C. Yenjai, Cytotoxicity against cholangiocarcinoma cell lines of zerumbone derivatives., Eur. J. Med. Chem. 45 (2010) 3794–802.

[318] S.I. Abdelwahab, A.B. Abdul, N. Devi, M.M.E. Taha, A.S. Al-zubairi, S. Mohan, A.A. Mariod, Regression of cervical intraepithelial neoplasia by zerumbone in female Balb/c mice prenatally exposed to diethylstilboestrol: involvement of mitochondria-regulated apoptosis., Exp. Toxicol. Pathol. 62 (2010) 461–9.

[319] N.A. Samad, A.B. Abdul, R. Abdullah, T.A.T. Ibrahim, H. Rahman, Y.S. Keong, Zerumbone (ZER) induces apoptosis in HEPG2 cells via mitochondrial pathway, Int. J. Pharm. Pharm. Sci. 7 (2015) 298–302.

[320] A. Murakami, H. Ohigashi, Y. Ikeda, M. Kizaki, Zerumbone, a bioactive sesquiterpene, induces G2/M cell cycle arrest and apoptosis in leukemia cells via a Fas- and mitochondria-mediated pathway, Cancer Sci. 98 (2007) 118–126.

[321] Y. Ding, S. Su, W. Tang, X. Zhang, S. Chen, G. Zhu, J. Liang, W. Wei, Y. Guo, L. Liu, Y.-G. Chen, W. Wu, Enrichment of the β-catenin-TCF complex at the S and G2 phases ensures cell survival and cell cycle progression., J. Cell Sci. 127 (2014) 4833–45.

[322] P. Kaldis, M. Pagano, Wnt Signaling in Mitosis, Dev. Cell. 17 (2009) 749–750.

[323] H.S. Rahman, A. Rasedee, C.W. How, A.B. Abdul, N.A. Zeenathul, H.H. Othman, M.I. Saeed, S.K. Yeap, Zerumbone-loaded nanostructured lipid carriers: preparation, characterization, and antileukemic effect., Int. J. Nanomedicine. 8 (2013) 2769–81.

[324] S. Zhang, Q. Liu, Y.Y. Liu, H. Qiao, Y.Y. Liu, Zerumbone, a Southeast Asian Ginger Sesquiterpene, Induced Apoptosis of Pancreatic Carcinoma Cells through p53 Signaling Pathway., Evid. Based. Complement. Alternat. Med. 2012 (2012) 1–8.

[325] M. Sharma, C. Jamieson, M. Johnson, M.P. Molloy, B.R. Henderson, Specific Armadillo repeat sequences facilitate β-catenin nuclear transport in live cells via direct binding to nucleoporins Nup62, Nup153, and RanBP2/Nup358, J. Biol. Chem. 287 (2012) 819–831.